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Autologous Fat Transplantation to the Female Breast after Surgery and Radiotherapy
Assessment of Patient-Reported Outcomes, Radiology and Gene Expression Patterns

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"Deeds. Not words."

- Emmeline Pankhurst, suffragette

Till mina pojkar

Autologous Fat Transplantation to the Female Breast after Surgery and Radiotherapy
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ABSTRACT

Autologous fat transplantation (AFT) is commonly used as a complementary method to enhance breast appearance after breast surgery, radiotherapy and breast reconstruction. Some of the indications for AFT to the breast are contour irregularities, volume deficits, chronic wounds, soft tissue indurations and scarring. Although AFT is well established with the primary aim with AFT is to enhance the patient's satisfaction, research on patient perceptions is sparse. In the 1980s the American Society of Plastic Surgeons (former American Society of Plastic and Reconstructive Surgeons) expressed the concern that AFT could lead to false-positive radiological findings that subsequently would lead to excessive examinations. In 2009 they requested more studies on AFT to breast cancer patients. Apart from using autologous fat as a filler and to soften the breast tissue, AFT can be used to mitigate radiation-induced fibrosis and dermatitis. The effect of AFT on radiation-damaged tissue is well described clinically, but the biological processes behind the effects are still unknown. These issues were also studied in the present research.

A retrospective study (I) was carried out on 43 patients using a study-specific questionnaire to assess their perceptions of the results of AFT. Post-operative complications were also recorded. The patients reported overall good results and only one minor complication was found in all patients.

In a prospective case-series (II) patient-reported outcomes were assessed in 48 patients using a study-specific questionnaire and the 36-Item Short Form Health Survey (SF-36), before, and up to two years after AFT. Sixteen of the 20 items in the questionnaire were significantly improved two years post-operatively, including pain, scars, appearance and softness of the breast, as well as inclination to perform physical activities in public areas. The scores obtained with SF-36 concerning health related quality of life did not change after AFT, and did not differ from a Swedish reference population.

In the third study (III), 44 patients underwent mammography and ultrasound before and one year after AFT. Assessment was carried out by experienced radiologists. No difference in the overall assessment (BI-RADS score 2) could be seen. AFT was found to significantly increase the number of breasts with oil cysts, where the breasts with oil cysts had been transplanted with larger volumes of fat.

Finally, gene expression in adipose tissue was investigated with microarray technique and Hallmark gene set enrichment analysis (IV). Biopsies were taken from the adipose tissue of the irradiated breast and the contralateral non-irradiated breast of ten women before AFT. Biopsies were also taken bilaterally one year after AFT of the irradiated breast. Among the 3000 most differentially expressed genes comparing irradiated and non-irradiated biopsies before AFT, 45 enriched pathways were found. After AFT to the irradiated breast, 575 of the 3000 previously differentially expressed genes were reversed in the irradiated and AFT treated adipose tissue, and thus affected by AFT. Among these 575 genes, 13 pathways were identified, all of them also found in the pre-operative analysis. The leading canonical pathways in the two analyses were interferon gamma response, hypoxia and epithelial mesenchymal transition.

The conclusions drawn from these studies are that AFT was perceived as a good complementary method of treatment by the patients, who experienced improvements in several aspects, and that AFT has no or little negative effects on post-operative radiological assessment. Furthermore, may AFT reverse differential gene expression in genes involved in inflammation, hypoxia and fibrosis that could have been caused by radiotherapy.

LIST OF SCIENTIFIC PAPERS

I. Schultz I, **Lindegren A**, Wickman M.
Improved shape and consistency after lipofilling of the breast: patients' evaluation of the outcome
J Plast Surg Hand Surg 2012: 46; 85-90

II. **Lindegren A**, Schultz I, Wickman M.
Improved Patient-Reported Outcomes after Autologous Fat Transplantation and corrective surgery after Breast Surgery
Submitted

III. **Lindegren A**, Wickman M, Bygdeson M, Azavedo E, Schultz I.
Autologous Fat Transplantation to the Reconstructed Breast Does not Hinder Assessment of Mammography and Ultrasound: A Cohort Study
World J Surg 2016: 40; 1104-1111

IV. **Lindegren A**, Schultz I, Sinha I, Cheung L, Kahn A.A, Tekle M, Wickman M, Halle M.
Autologous fat transplantation alters gene expression patterns related to inflammation and hypoxia in the irradiated breast
Br J of Surg *Accepted November 2018*

CONTENTS

LIST OF ABBREVIATIONS

3D	Three-Dimensional
AFT	Autologous Fat Transplantation
AT-MSC	Adipose Tissue Derived Mesenchymal Stem Cells
ASPS	American Society of Plastic Surgeons
BCCT.core	Breast Cancer Conservation Treatment. cosmetic results
BCS	Breast-Conserving Surgery
BI-RADS	Breast Imaging Reporting and Data System
BRCA	Breast Cancer Gene
CD68	Cluster of Differentiation 68
CT	Computerized Tomography
CTGF	Connective Tissue Growth Factor
DIEP	Deep Inferior Epigastric Artery Perforator
EMT	Epithelial Mesenchymal Transition
FC	Fold Change
FDR	False Discovery Rate
FNAC	Fine-Needle Aspiration Cytology
Gy	Gray (unit of ionizing radiation dose)
HRQoL	Health Related Quality of Life
IHC	Immunohistochemistry
INF	Interferon
LD	Latissimus Dorsi
LENT-SOMA	Late Effects Normal Tissue
	Subjective, Objective, Management, and Analytic
LRR	Loco-Regional Recurrence
MCS	Mental Component Summary
MMRM	Mixed Effect Model Repeat Measurement
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
OT	Ordinal Trend
PCS	Physical Component Summary
PREM	Patient Reported Experience Measures
PRO	Patient Reported Outcome
PROM	Patient Reported Outcome Measures
RD	Radiodermatitis

RIF	Radiation Induced Fibrosis
RIN	RNA Integrity Number
ROS	Reactive Oxygen Species
RT	Radiotherapy
SF-36	36-item Short Form Health Survey
SSQ	Study-Specific Questionnaire
SVF	Stroma-Vascular Faction
SweBCG	Swedish Breast Cancer Group
TRAM	Transverse Rectus Abdominis Myocutaneous
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor

1 THE HISTORY OF AUTOLOGOUS FAT TRANSPLANTATION

Various terms have been used to describe autologous fat transplantation (AFT) over the years, including lipofilling, lipomodelling, fat grafting and fat transfer. The history of AFT dates back to the late 19th century, when the German surgeon Gustav Adolf Neuber (founder of the first aseptic hospital) injected adipose tissue to treat scars in the eye region. Many attempts were subsequently made to transplant small fat biopsies underneath the skin, but with poor results due to reabsorption and fibrosis (1). In 1983, Illouz described a technique in which fat tissue was harvested by suction-assisted lipectomy (2). Bircoll described a similar method of liposuction in 1987, and claimed to have taught Illouz the technique in 1984 (3, 4). However, reabsorption continued to be a significant problem. A decade later, Sidney R. Coleman described a technique in which the lipocytes were better preserved by harvesting under a low negative pressure, centrifugation of the aspirate to remove oil and blood, and injection of the lipocytes in multiple layers and narrow tunnels to enhance the diffusion of nutrients into the transplants (5-7). This technique is still the dominant one today, although some modifications have been made to fat harvesting and purification. However, in 1987, the American Society of Reconstructive and Plastic Surgeons (today American Society of Plastic Surgeons, ASPS) expressed the concern that the use of AFT in breast augmentation would lead to scarring and calcification that could lead to difficulties in detecting early breast cancers or false-positive findings in mammography, leading to increased breast biopsies being performed for benign conditions (8). This started a debate on the safety of AFT in the breast, but surgeons continued to use and study the method. In 2009, the ASPS published a new report. Their conclusion regarding safety was; "based on a limited number of studies with few cases, there appears to be no interference with breast cancer detection; however, more studies are needed to confirm these preliminary findings". In the light of this, the work described in this thesis was initiated.

2 BACKGROUND

2.1 BREAST CANCER AND ITS TREATMENT

Considerable developments have taken place in the treatment of breast cancer. In the late 19th century, William Halsted performed radical mastectomies removing the whole breast, axillary lymph nodes, and also the underlying pectoral muscles (9). This remained the predominant method, with minor adjustments, until 1972, when John Madden described modified radical mastectomy, in which both pectoral muscles were preserved (10). This technique is still used today. Breast-conserving surgery (BCS) was developed in parallel, and is since the late 20th century a well-established and safe technique when used in combination with post-operative radiotherapy. BCS has also developed, and today oncoplastic techniques are commonly used to optimise the aesthetic outcome. In addition to surgery, medical treatment has steadily gained ground and is now an integral part of breast cancer treatment.

During the 1980s, guidelines for breast cancer treatment were formulated in most of the health care regions in Sweden. In 2000, the Swedish Breast Cancer Group (SweBCG) developed the first evidence-based national guidelines which, in a later version (2014), were adopted as the Swedish National Breast Cancer Management Programme (11). This 300-page document is continuously revised by leading researchers and clinicians to ensure that it is always up to date and that new evidence is introduced.

2.1.1 Breast cancer epidemiology and prognosis

Breast cancer is the most common cancer affecting women worldwide. In 2016 the incidence was 152.7 per 100 000 women in Sweden, and it is increasing yearly (12, 13). The prevalence in 2016 was 105 825 (women) (13). The prognosis is good in many countries, but varies globally (11, 14, 15). In Sweden, the 5-year survival is 83%, and was unchanged for all patients diagnosed between 2009 and 2012 (16).

2.1.2 Diagnosis

2.1.2.1 The Swedish screening programme

The Swedish National Board of Health and Welfare recommends that all Swedish women aged 40-74 should be offered mammography every 18-24 months as part of a national breast cancer screening programme (17). The screening programme is considered to reduce breast cancer mortality by 15-25% in the whole population, and even more in those participating in the screening programme (11, 18, 19). However, screening for breast cancer is not uncontroversial, and it has been criticised for overdiagnosis (19).

2.1.2.2 Triple assessment

When a woman presents with a lump in her breast, it is investigated by triple assessment which comprises clinical examination, biopsy and imaging. A clinical breast examination involves a systematic physical examination of the chest, including breasts, nipples, axillae and the supraclavicular spaces. In Sweden, fine-needle aspiration cytology (FNAC) is standard in the investigation of breast lumps, and in most cases this is deemed sufficient pre-operatively if the other two cornerstones in the triple assessment are unambiguous. If the diagnosis is uncertain, further investigation of the tumour with a core needle biopsy is recommended (11). If neoadjuvant treatment is considered core needle biopsy is required.

Radiological examination combined with cytology is the most important investigation. The sensitivity is 85-90% but decreases with increasing density of the breast since a higher density makes the detection of a tumour more difficult (11, 20). High-density breasts also have a higher risk of cancer (21). The natural development of breast tissue is from a high density (glandular tissue) in young fertile women, to lower density (fatty tissue) in postmenopausal women. In Sweden, a system similar to the Breast Imaging Reporting And Data System (BI-RADS) (22) is used to classify and code the images and findings on a five-level scale (Table 1). BI-RADS can also be used to determine a score for the entire mammogram and to classify the density of the breast. Clinical mammography also plays a major role in the surveillance after breast cancer treatment. Ultrasound is the method of choice in younger women (<30 years), or if the woman is breast-feeding or pregnant. It is also widely used as a complement to mammography, especially when the mass is non-palpable. As in all ultrasound examinations, the specificity is highly user dependent. In Sweden, magnetic resonance imaging (MRI) is generally used only in special cases, for example, if the patient has a palpable mass but no mammography or ultrasound findings, or in patients with a high hereditary risk of breast cancer. Galactography can be used to examine the milk ducts if the patient presents with nipple discharge.

Table 1. Scoring system used in Sweden for classification of images of the breast

Code	Interpretation
1	Negative/Normal breast
2	Benign finding
3	Probably benign finding
4	Suspicion of malignant finding
5	Radiological malignant finding

2.1.3 Treatment

2.1.3.1 Systemic treatment

Chemotherapy can be given in addition to surgery to improve survival. Historically, it has been given as an adjuvant treatment, but this practice has changed over recent years. Neoadjuvant chemotherapy is recommended if the patient presents with locally advanced tumours. It can also be given to shrink a resectable tumour, thereby avoiding mastectomy. In 2015, 19% of chemotherapy was neoadjuvant, although the variation was considerable (4-26) throughout the country. Depending on the protocol, a 1.4-13% absolute reduction in breast-cancer-specific mortality has been reported (11).

All patients with oestrogen receptor positive (ER+) carcinomas (about 85%) should be offered anti-hormonal treatment. Tamoxifen is a selective oestrogen receptor modulator, and has been shown to reduce the absolute risk of recurrence and breast-cancer-specific mortality by 13.2 and 9.2%, respectively after 15 years when administered for five years (23). Tamoxifen can be given to pre- as well as postmenopausal women. Aromatase inhibitors on the other hand, reduce the non-ovarian oestrogen production in the body, and are therefore only effective in postmenopausal women whose ovarian oestrogen production has ceased. Aromatase inhibitors have a slightly better effect on both recurrence and mortality than tamoxifen in this group (11). Ovarian suppression can be obtained either by surgery, or by repeated injection of a hormone agonist, the latter only having an effect in premenopausal women. The effect on survival and recurrence has not been fully investigated, but depends on several factors, including other therapies (11, 24, 25).

Trastuzumab is an antibody that binds to the HER2 oncogene receptor, which is expressed on tumour cells in approximately 15% of breast cancer tumours. It has been estimated that the 3-year overall survival and recurrence-free survival can be increased by 9 and 11%, respectively, when combined with adjuvant chemotherapy (11).

2.1.3.2 Breast surgery

Historically, mastectomy was the gold standard. In Sweden, the current recommendation for mastectomy is unifocal or multifocal tumours that cannot be radically excised with cosmetically good results. Mastectomy is also recommended if the patient declines BCS, if there are contraindications to RT, or if the patient has a local recurrence and has previously been treated with BCS and RT (11). All methods of breast surgery are under continuous development, and during recent years nipple-sparing mastectomy has been used more frequently in selected patients without jeopardizing oncological safety (26). Today, the most common surgical method in Sweden is BCS. This is a method in which only the part of the breast containing the tumour is excised. In 2017, 83% of Swedish patients with small invasive breast cancer tumours without distant metastases underwent BCS (16). This method requires RT to reduce the risk of local recurrence (11, 27). It has been shown that the patient's HRQoL is better after BCS than after mastectomy, and that more radical excision seems to have a negative impact on the patient's satisfaction with the outcome (28, 29).

Techniques that combine oncological safety and reconstructive plastic surgical methods to achieve a good post-operative aesthetic result are called oncoplastic breast surgery. These techniques allow the excision of larger tumours without risking the patient's safety or the aesthetic result. These methods can be regarded as a third alternative when BCS is not appropriate and mastectomy is not necessary (30), and are carried out at the specialist breast units in Sweden. A common method is to combine BCS with a contralateral breast reduction to achieve better post-operative symmetry. Frequently used techniques for tumour excision include round block, or various techniques for reduction mammoplasty (29, 31, 32). Other examples are batwing mastopexy and abdominal advancement flaps (33, 34).

Women with a significantly increased risk of breast cancer can be candidates for bilateral risk-reducing mastectomy (35, 36). The indications for surgery are being a carrier of the breast cancer genes (BRCA) BRCA-1 or BRCA-2, or having a family history indicating an autosomal dominant inheritance of breast cancer (11).

In addition to breast surgery, sentinel lymph node biopsy is performed in women with invasive cancer without any signs of axillary metastasis. The sentinel lymph node is the lymph node (nodes) that is first in the node chain to drain the breast tumour. It is normally located in the ipsilateral axilla, but can be located parasternally in the internal mammary chain. The latter is not routinely explored as it is surgically demanding and leads to additional scars and morbidity. A blue dye and a radioactive isotope (lymphoscintigraphy) are injected either superficially into the subareolar plexus over the tumour, or deep into the glandular tissue (37). Sentinel nodes are identified visually by the injected dye and acoustically by the signal from a gamma probe. The surgeon extirpates the nodes that are blue, are identified by the gamma probe, or have pathological features. The Swedish National Breast Cancer Management Programme recommends the extirpation of a maximum of four nodes. The nodes are sent for histopathological examination. Supplementary axillary surgery may be required if the tumour has metastasised. Sentinel node biopsy indicates the cancer stage.

2.1.3.3 Radiotherapy

Radiotherapy (RT) is recommended for almost all women who have undergone BCS to erase microscopic tumour foci, which may remain in the breast. RT reduces the risk of local recurrence to an acceptable level compared to mastectomy. The absolute reduction in the 10-year risk of first recurrence is 15.7%, and the reduction in the 15-year risk of breast-cancer-specific death is 3.8% (38). Previously no difference in overall survival has been found between BCS with or without RT, and mastectomy (27). However, in a multicentre study it has recently been shown that BCS and RT was superior to mastectomy regarding both overall survival and breast-cancer-specific survival (39). Depending on tumour stage, RT can also be recommended in mastectomised patients to reduce the risk of recurrence. However, the evidence for RT in mastectomised patients is not as strong as for those undergoing BCS. The irradiation dose and target of irradiation have also been modified over the years. The current standard dose in Sweden for N0 tumours (no metastases) is 40 Gy in 15 fractions, given as five fractions per week. Additionally, patients ≤50 years old receive a 10-16 Gy boost, at 2 Gy per fraction. The dose for pN+ tumours is generally 46-50 Gy in 25 fractions. Women ≤50 years also receive an additional 10-16 Gy boost after BCS (11).

Side effects of radiotherapy – radiodermatitis and fibrosis

RT has been part of cancer treatment since the beginning of the 20th century, and is an important part of breast cancer treatment. Its side effects are well known, and range from increased mortality from lung- and heart disease (11, 38) to systemic effects such as fatigue and sleep problems to local symptoms (40, 41). The severity of the side effects varies with dose, the number of fractions and the area treated, as well as intrinsic properties such as connective tissue diseases (42). In addition, physical reactions may increase over time (43).

Radiodermatitis is a common problem. Acute symptoms appear within 90 days of initiating therapy, and normally resolve in a few weeks. Nearly all patients exhibit acute skin reactions resulting from RT, but about half of the patients report skin reactions and pain up to six months after treatment (40). The symptoms can be very mild, such as erythema, and increase with irradiation dose, including dry skin, hair loss, dry desquamation, moist desquamation and even ulceration (41). Chronic radiodermatitis can appear early or up to years after completing RT. Clinical manifestation of chronic radiodermatitis includes atrophy, telangiectasia, pigmentation changes, radiation-induced fibrosis (induration and thickening of the dermis), ulceration and necrosis (44).

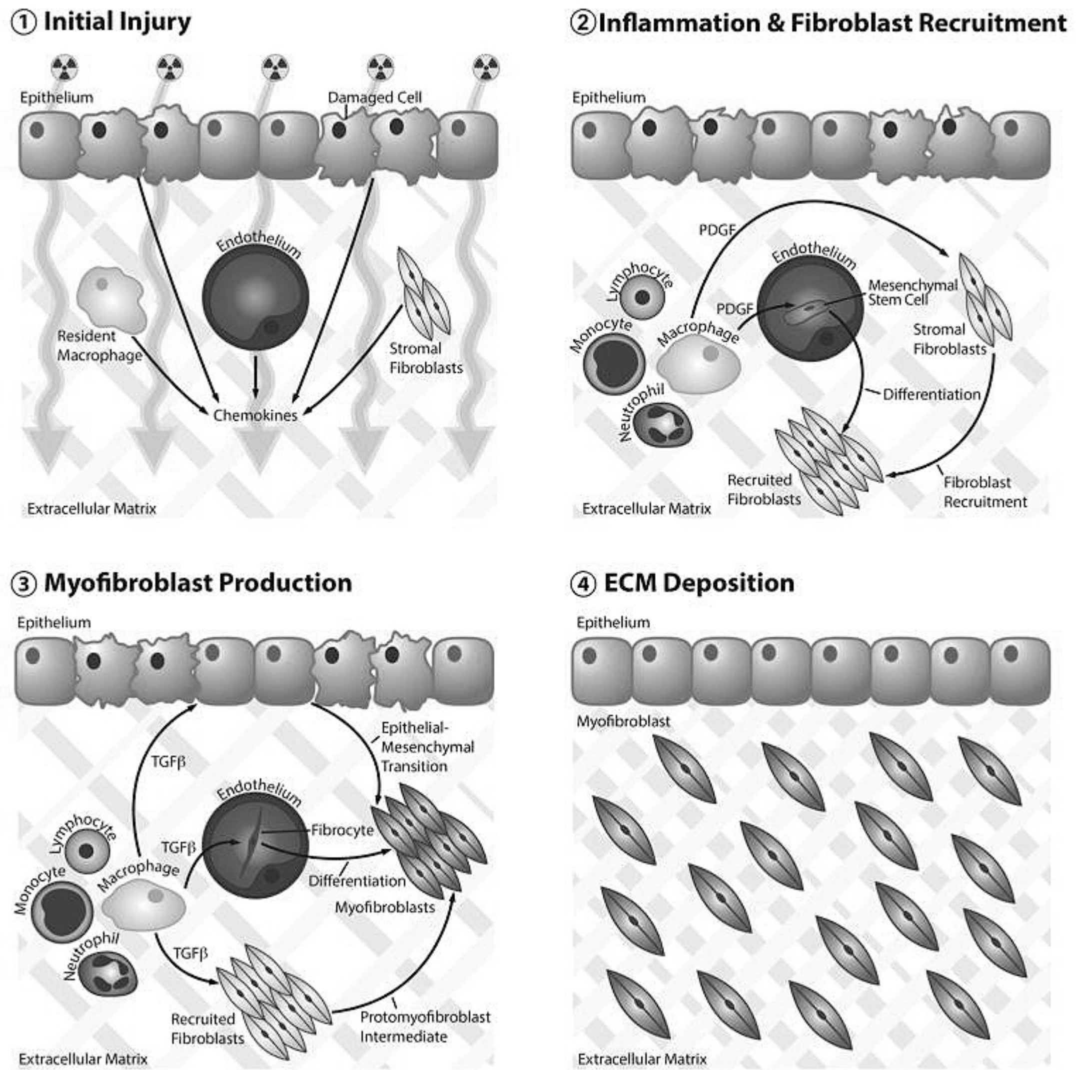

Figure 1. Pathogenesis of radiation-induced fibrosis. 1) Cell damage caused by ionizing radiation leads to the outflow of chemokines. 2) Neutrophils, lymphocytes and monocytes migrate to the site. Macrophages recruit stromal fibroblasts and stimulate differentiation of circulating mesenchymal stem cells to fibroblasts. 3) Macrophages initiate differentiation of circulating fibrocytes through epithelial mesenchymal transition, and development of the recruited and differentiated fibroblasts into myofibroblasts. 4) The myofibroblasts proliferate and surplus deposition and decreased degeneration of extracellular matrix results in fibrosis with reduced vascularity and a lack of cells.
(Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Journal of Cancer Research and Clinical Oncology, Radiation-induced fibrosis: mechanisms and implications for therapy, Straub J, et al., Copyright Clearance Center's RightsLink® 2015)

Radiation-induced fibrosis (RIF) is the main cause of morbidity after RT in all organs. A wide range of genetic variations can predispose a patient to RIF, and this may be the cause of sustained pathology in the otherwise healthy tissue surrounding the treated tumour area. However, the underlying biological reactions are not fully understood (45). A schematic overview of the RIF process is illustrated in Figure 1. A serious reduction in proliferating cells and a significant increase in apoptotic stromal vascular faction (SVF) cells, as well as a reduction in the proliferation and differentiation ability of non-haematopoietic progenitors of the SVF in irradiated subcutaneous fat in mice have been shown (46). Irradiation also affects a wide range of cellular structures directly by disrupting them. As a result, the cells release chemoattractants that induce inflammation. Hypoxia is considered a distinct feature of RIF and is caused by vasculopathy and is also correlated to perivascular matrix deposition and induction of extracellular matrix synthesis (47-52). Vasculopathy and hypoxia intensify tissue damage and inflammation. Neutrophils are the quickest to respond to damaged cells and further exacerbate the inflammatory response by releasing chemokines, cytokines and growth factors. Cytokines promote the differentiation of macrophages that recruit fibroblasts, directly and indirectly via differentiation of mesenchymal stem cells (MSC). Furthermore, macrophages stimulate the differentiation of fibroblasts to myofibroblasts directly and through epithelial mesenchymal transition (EMT). Myofibroblasts produce excess collagen, fibronectin and proteoglycans, leading to fibrosis and rigidity of the tissue. The immense collagen production reduces vascularity and causes ischaemia, which may lead to loss of function, atrophy and necrosis (42, 53). In parallel with this, irradiation also causes intracellular generation of reactive oxygen species (ROS), which can initiate a series of reactions causing damage to DNA, proteins and lipids. The release of ROS disturbs the normal oxidative metabolism, giving rise to associated chronic inflammatory responses. The formation of free radicals occurs not only immediately after irradiation, but can continue for several months. Oxidative reactions take place not only in non-irradiated neighbouring cells, but surprisingly, also in the cells' progenies and the neighbouring cells' progenies, causing long-lasting damage (54). The inflammatory response and ischaemia that cause tissue damage are often compared with chronic inflammation and a disturbed wound healing process (42, 53, 55, 56). Chronic inflammation, fibrosis and the ischaemic environment make the tissue vulnerable, and are important factors contributing to the clinical effects of RT described above, that make surgery more challenging.

RT and chronic radiodermatitis are known to have a negative impact on the complication rate, reconstruction failure rate and aesthetic result after breast reconstruction (57-59). Contour differences, breast oedema, capsular contracture and mammillary deviation are common complications (60, 61). It has been shown that patients who have undergone RT report poorer outcome and satisfaction after breast reconstruction than those who have not. Patients who have undergone RT may also experience reduced psychosocial well-being and sexual well-being over time (62). In contrast, a meta-analysis of pooled data from observational studies on women who had undergone immediate autologous breast reconstruction with or without post-operative RT, revealed no significant difference in complication rate or revisional surgery. However, the fat necrosis rate and loss of volume was higher in patients who had received RT (63). Longo et al. found that irradiated patients needed significantly more AFT procedures with smaller fat volumes as a result of post-irradiation conditions than non-irradiated patients undergoing total breast reconstruction with AFT. The total aesthetic score and skin texture were also superior in the non-irradiated group (64). RT thus seems to have a negative impact on the efficiency of AFT.

2.2 BREAST RECONSTRUCTION

History of breast reconstruction started before Halsted's mastectomies, with attempts to correct breast deformities using both flaps and autologous transplantation of lipomas to the breast (65). The real breakthrough came in 1964 with the first silicone implant (66). A few years later, the predecessor to the modern expander prosthesis was invented (67), and in the 1980s Becker introduced detachable inflation domes (68) and the expander could thus be left in place. Initially, the implant was placed subcutaneously or pre-pectorally, but the technique was modified at the beginning of the 1980s due to high rates of capsular contracture, and the implant has since then been placed sub-muscularly, with better results. Recently, some surgeons have argued to again place the implant pre-pectorally in selected patients (69). Different implant surface textures have been tried to avoid capsular contracture, with various results (70). Many local flaps have been tried, and the use of abdominal tissue was first described in 1979 (65). Development has been rapid towards more complicated methods, and today free flaps and microsurgical procedures are frequently used to create new breasts. AFT is an important tool in breast reconstruction to further enhance the outcome, usually as a complementary procedure to achieve a better result.

According to European guidelines, "a unit team must provide breast surgical reconstruction" for patients not suitable for BCS and patients with extensive local disease (71). Reconstruction can be performed either immediately after mastectomy or at a later stage (delayed). Between 2014 and 2017, 30% of all mastectomised patients in Stockholm underwent immediate reconstruction (16), the national rate was 11%, but is slowly increasing (16, 72). Delayed breast reconstruction is more common, and is carried out about 1-2 years after the initial cancer surgery. Delayed reconstructions are not registered in Sweden and hence no rates are available. The indication for delayed reconstruction is the patient's wish. A breast reconstruction can make life easier (reducing problems associated with poor fitting of clothes or an external prosthesis), and improve the patient's satisfaction with her breasts, together with her sexual and physical well-being (73, 74).

2.2.1 Implants

Depending on skin access and remaining breast volume, either a permanent implant or an expander (adjustable) implant, can be used (Figure 2). The implant is usually placed in a sub-muscular pocket created by releasing the pectoralis major and serratus anterior muscles. Expander implants are frequently used in breast reconstruction. This involves a two-stage procedure in which the expander is inserted during the first operation. The expander, and thus the tissue, is then gradually expanded with saline via a subcutaneous injection dome over a period of several weeks. The expander can be replaced by a permanent prosthesis when expansion is completed. Permanent expanders can also be used, and then only the injection dome is removed after expansion. Breast reconstruction with implants has several advantages. In contrast to flaps, there is no problem associated with skin colour matching, no donor site morbidity, and the sensibility is better preserved than with free flaps. The method may be done as day surgery, but more often as an inpatient procedure requiring a few days hospital stay. The disadvantages are a risk of capsular contraction (cumulative incidence 8-16% in 3-6 years), which can be more than doubled in combination with irradiation (which is a relative contraindication), and a high risk of reoperation (cumulative incidence 27-52% in 3-6 years) (61, 75, 76). The breast will not be as soft as the body´s own tissue, and there is a risk of a less natural appearance (61). Additional surgery is often required on the contralateral breast to obtain better symmetry. AFT can be used to correct rippling and irregularities caused by the implant. Implants can be combined with other types of autologous reconstruction methods, and can be used in both immediate and delayed reconstructions. Complementary methods using an acellular dermal matrix or autologous dermal grafts are sometimes used to create a better implant pocket (77).

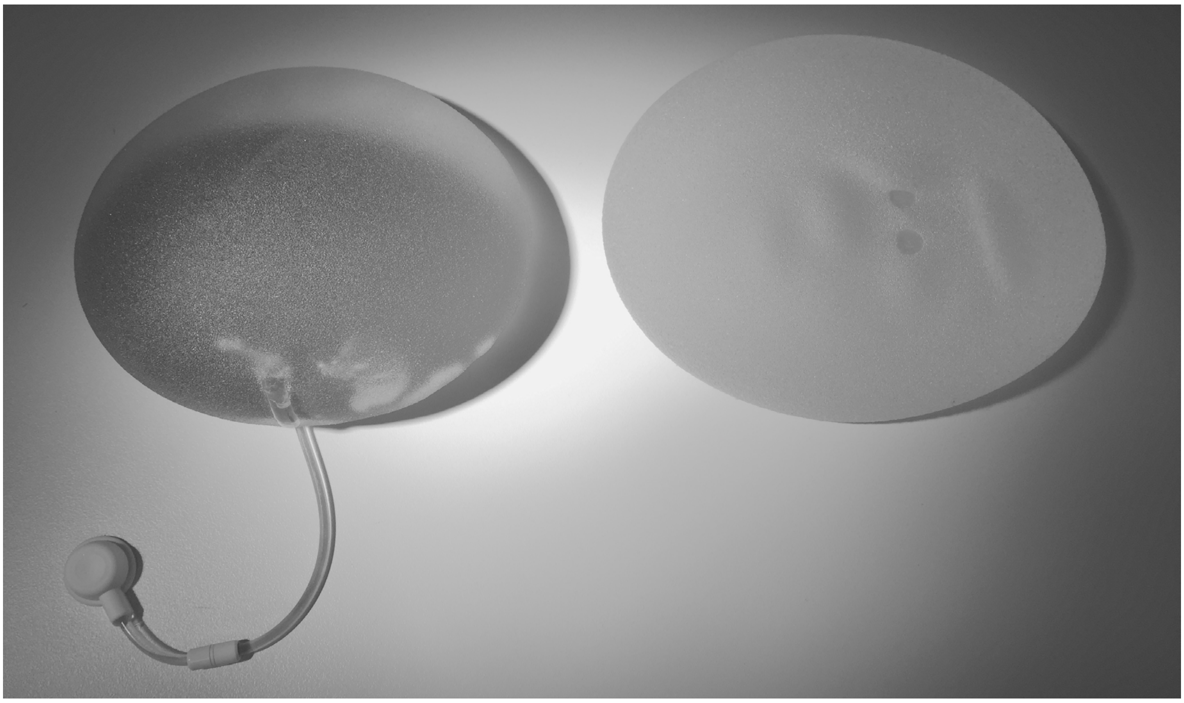

Figure 2. Breast prostheses. Expandable prosthesis with injection dome to the left. The injection dome is placed subcutaneous Permanent prosthesis to the right. (Anna Lindegren 2018)

2.2.2 Flaps

There are two major types of flaps; pedicled and free flaps. Pedicled flaps are connected to the donor site by blood vessels and often nerves. In the case of free flaps, blood vessels, and sometimes nerves, are sutured to recipient vessels and nerves, at the reconstruction site. Flaps used in breast reconstruction consist of skin, subcutaneous fat, fascia and muscles in various combinations. Flaps are used to provide volume, surface and healthy tissue to a breast with tissue deficiencies, irradiated tissues and implants in need for soft tissue coverage.

Small pedicled flaps are usually used to add soft tissue to cover implants. The surgical procedure is less complex than that with free flaps, and the donor site scar is often less prominent (78). The latissimus dorsi (LD) flap is often used for reconstruction of irradiated breasts. The LD flap was first described in 1896 by the Italian surgeon Iginio Tansini, but became widely known after being re-described by Olivari in 1976 (79). The LD flap is a musculocutaneous flap in which part of the LD muscle is dissected together with subcutaneous fat and often a skin island. It is rotated and tunnelled with intact blood supply, and sometimes also nerves, and placed in the lower pole of the breast. An LD flap is usually combined with implants and, in recent years, also with AFT (80, 81). The lateral thoracodorsal flap (in Sweden called the "Gothenburg flap") is a fasciocutaneous flap in which tissue from a horizontal triangle extending laterally is transposed vertically, the base being in the anterior axillar line, to add tissue to the breast. An implant is placed sub-muscularly (82). This flap can be useful in some cases, although it is not frequently used. The transverse rectus abdominis myocutaneous (TRAM) flap is an abdominal flap based on the deep inferior epigastric vessels and is raised with an elliptic incision from hip to hip in the lower part of the abdomen. The TRAM flap contains skin, the subcutaneous fat layer and the rectus abdominis muscle or part of the muscle (muscle-sparing TRAM flap). The pedicled flap is tunnelled under the skin to the chest. When the donor site is closed the umbilicus must be transpositioned (83). The advantages of the TRAM flap are the possibility to create a whole new breast with a natural shape, which varies in size if the patient gains or loses weight, and the breast feels natural. Pedicled TRAM flaps are seldom used in Sweden due to abdominal wall donor site morbidity and muscle weakness.

A range of free flaps with different origins (abdominal, gluteal and thigh etc.), can be used in breast reconstruction; the most frequently used in Sweden being an abdominal flap, the deep inferior epigastric artery perforator (DIEP) flap. The DIEP flap is based on the deep inferior epigastric vessels, which can be anastomosed to the internal mammary vessels in the chest (which requires microsurgery) sometimes after a small portion of rib and cartilage is excised, or to a vessel in the axilla (84). The superficial inferior epigastric artery flap is based on the superficial inferior epigastric vessels, as the name implies. Both flaps contain skin and the subcutaneous fat layer, and are raised in the same way as the TRAM flap (which can also be a free flap) with an elliptical incision and umbilicus transposition, but with preservation of the rectus muscle. The advantages are the same as with the pedicled TRAM flap, but without muscle weakness, and there is no need for tunnelling under the skin. Unfortunately, most of the sensibility is lost with free flaps as the nerves are cut.

2.3 METHODS OF EVALUATING THE RESULTS OF BREAST RECONSTRUCTION

2.3.1 Patient-reported outcome

With the evolution of modern health care, the patient's rights and participation in decision-making have been strengthened. It is now mandatory in Sweden to involve patients in their treatment. Patient-reported outcome measures (PROMs), self-assessment surveys assessing patient-reported outcome (PRO), are important instruments to measure patients' perceptions of their symptoms and the outcome of their treatment. It has been shown that using PROMs in clinical practice improves patients' satisfaction with care and PRO (85). The PRO is best measured using questionnaires to rate symptoms, functioning level, health-related quality of life (HRQoL) and sometime also satisfaction with the care given and the care provider. Evaluation of the last two is referred to as the patient-reported experience measure. PRO can be measured on different levels from general HRQoL to diagnosis-specific symptoms.

2.3.1.1 SF-36

The 36-item Short Form Health Survey (SF-36) was developed at the end of the 20th century in English, and became available in Swedish shortly after. It is used to measure health-related HRQoL. The questionnaire contains eight domains: physical functioning, physical role functioning, bodily pain, general health, vitality, social functioning, emotional role functioning and mental health. In the Swedish version of SF-36, a reference population consisting of 8 930 people who lived in seven regions, in 1991-92, is provided. According to national registers, those living in the specific urban area of the reference population (Gothenburg) have a lower HRQoL than other equally sized communities in Sweden. The scores of the SF-36 are values between 1 and 100 for each domain, where 100 indicates the highest possible HRQoL in that domain. In addition, two summary scores can be used: the physical component summary (PCS) and the mental component summary (MCS) scores (86, 87).

2.3.1.2 BREAST-Q

BREAST-Q was the first validated PROM to assess PRO in women who had undergone breast reconstruction (88, 89). It first became available in English in 2009. It was translated into Swedish in 2012, but has only been validated for North American women. The questionnaire consists of a pre-operative survey form and a post-operative survey form, containing questions on six domains: psychosocial well-being, physical well-being, sexual well-being, satisfaction with breasts, and satisfaction with outcome. Moreover, it also includes items on satisfaction with care. The scores are presented as a value between 1 and 100 for each domain, where 100 indicates the highest possible HRQoL for that domain.

2.3.1.3 Other PROMs

The most commonly used general questionnaires in Swedish national quality registries are SF-36, RAND-36 (90) and EQ-5D (91). RAND-36 is a free of charge questionnaire based on SF-36 with the same questions. RAND-36 is translated into Swedish (92). EQ-5D is an instrument for assessing general HRQoL in five domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. A new questionnaire for women undergoing breast reconstruction, QLQ-BRECON23, has recently[1] been developed and internationally validated by the European Organisation for Research and Treatment of Cancer (93). Most people are unknowingly familiar with Likert scales, which are widely used in surveys and questionnaires, and the possible responses are typically “strongly agree”, “agree”, “neutral”, “disagree” and “strongly disagree” or numbers for example 1-5. Opinions differ as to whether a Likert scale should be treated as an ordinal scale or an interval scale, or whether it is preferable to use mean values or medians when analysing the responses. The use of a Likert scale in a one-item survey is not recommended as it is considered not to be capable of assessing the hypothesis (94, 95).

2.3.2 Aesthetic outcome

Evaluating the aesthetic outcome is difficult and there is a high risk of subjectiveness regardless of how the assessment is carried out. In the light of PRO, it is debatable whether the opinion of an external assessor is of any interest at all (96). Moreover, it has been reported in a number of studies that there are discrepancies between the opinions of surgeons and patients, as well as between surgeons (78, 97-101). There are thus few analytical reports.

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1 E-published November 8 2017

When reporting the aesthetic outcome, tools such as the visual analogue scale, VAS, and three- to seven-point Likert scales are often used. Another option is to use ordinal rating scales, e.g. excellent, good, fair or poor. The drawbacks of using this type of scale are that the studies are non-reproducible, and their results are not comparable. Moreover, it is difficult to analyse the output, and there are different opinions as to whether the numerical values should be treated as ordinal or interval. The Breast Cancer Conservative Treatment. cosmetic results (BCCT.core) software offers a method based on the same principles as subjective ratings, and has been validated for BCS. The intention of this software is to provide an objective method for the assessment of the aesthetic outcome (102).

Anthropometry involves linear measurements between anatomical landmarks. In the case of breast surgery, these measurements are thought to facilitate pre-operative planning, in an attempt to obtain "normal" breasts after breast surgery or breast reconstruction. It can be used for pre- and post-operative comparison. The measurements were developed by Jack Penn in 1955, and were based on 20 women who were chosen among 150 volunteers because they had "aesthetically perfect breasts" (103). New "normal values" were published in 1986, based on 55 American women aged 18-31, and in 1997 measurements of "aesthetically perfect breasts" were reported for 50 Caucasian, nulliparous women aged 17-38 (104, 105). Anthropometric measurements can also be made on photographs. The advantages are that the image does not change, and retrospective evaluation is possible. On the other hand, some anatomical landmarks may not be visible on photographic images, and there are no widely used standardised protocols for this method.

It appears that we are moving towards objective measurements with three-dimensional (3D) measurements. Early measurements were performed with computerized tomography (CT) and MRI. The accuracy of these techniques was good, but they are time consuming for both the patient and the radiologist. In addition, MRI scans are expensive and CT entail radiation (106). Today, 3D surface imaging can be performed by laser scanning and by photography. Both methods rely on manually placed landmarks, and even small changes in position between imaging sessions make the calculations more difficult (107). The costs for 3D measurements are still high, but are decreasing (108).

3 AUTOLOGOUS FAT TRANSPLANTATION TO THE BREASTS

3.1 BACKGROUND

AFT is a widely used method for enhancing the aesthetic and functional outcome in various regions of the body. It can be used to rejuvenate areas of the face and hands, and to augment the breasts, gluteal region and genitals. However, it is also used to treat burn scars, chronic wounds, post-operative pain and radiation-damaged areas, and for the stabilization of vascular pedicles (109-115). In reconstructive plastic surgery, one main field of application of AFT is as a complement to, or as a part of, breast reconstruction after breast cancer surgery. It is minimally invasive and has low morbidity, and there is no scarring as is the case following reconstruction with flaps. Another positive side effect for many patients, is that liposuction is performed often on the abdomen or thighs. AFT is a technique with which the surgeon can correct minor flaws, as well as treat large areas of radiation-induced malformations.

Although AFT is a widely used method, many studies on AFT do not have a robust study design, the evidence level is low, and often statistically underpowered. Very few clinical trials have been conducted on AFT and few focusing on one particular area rather than many different. Moreover, most of the studies are not reproducible, and definitions of similar outcomes diverge. An explanation of the limitations may be that many surgeons have no incentive to properly assess AFT because of the positive features and the few surgical complications. Moreover, since AFT differs profoundly from other reconstructive methods and is difficult to compare with other methods, there is no truly suitable control group. A review of the current literature on AFT in breast reconstruction is given below.

3.2 SURGICAL TECHNIQUE OF AFT

The method comprises three stages: fat harvesting, fat preparation and fat injection illustrated in Figure 3. First, the donor site should be chosen. This is normally the location with the greatest excess fat tissue. Studies have been carried out to investigate the best donor site for AFT with regard to tissue viability, however, no differences were found (116, 117). A few studies have reported a higher yield of AT-MSC in fat harvested from the abdomen (118, 119). None of these studies considered the clinical outcome. The most common donor location is the abdomen; while the knee, thigh and flank are also frequent sites (120). The fat is harvested with either wet or dry technique. In the wet technique, a tumescent solution is infiltrated into the subcutaneous adipose tissue of the donator site; to provide local anaesthesia and to facilitate fat removal (121). In the dry technique the infiltration is not performed (122). No substantial differences in adipocyte viability have been reported between the techniques, however, it has been suggested that the wet technique may offer a slight improvement in cell viability, but there is no consensus (116, 123). Harvesting can be performed by syringe aspiration, suction by hand or with suction-assisted lipectomy, all of which have been reported to have comparable adipocyte viability (116, 123). The harvested fat is a mixture of viable cells, oil, blood and, when used, tumescent solution.

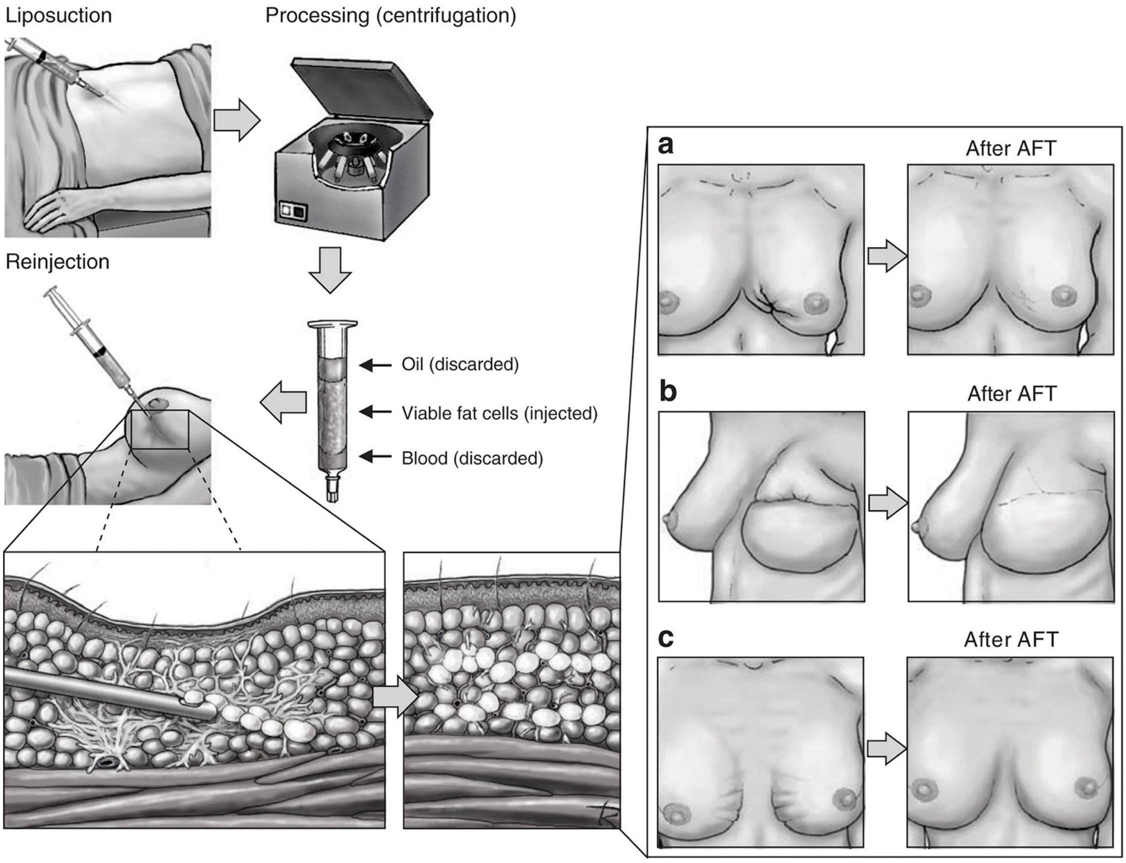
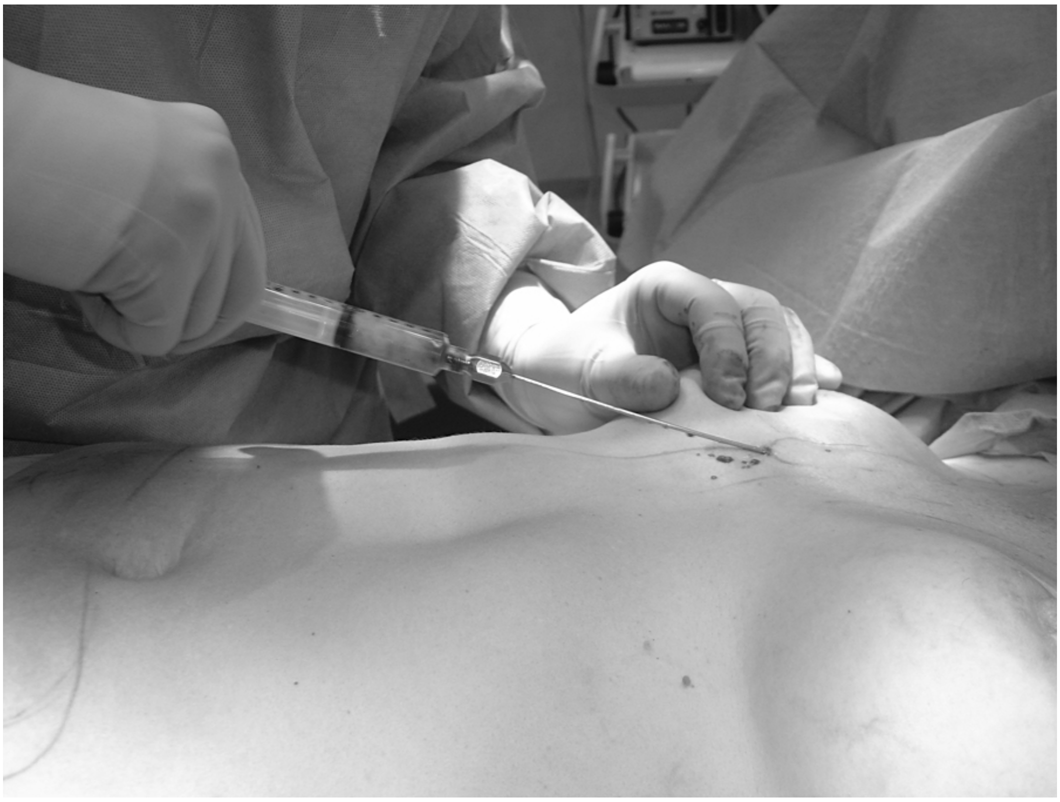


Figure 3. Autologous fat transplantation (AFT). The technique includes three steps: harvesting by liposuction; processing; and injection into the recipient area. a–c examples of the spectrum of indications for AFT treatment. a) Scar retraction after breast conserving surgery and sometimes radiotherapy. b) AFT after reconstruction with flap. b) Rippling over implants. *(Reprinted by permission from Wiley Online Library, The British Journal of Surgery. Meta-analysis of the oncological safety of autologous fat transfer after breast cancer. Krastev TK, et al. Copyright Clearance Center's RightsLink®, 2018)*

The harvested material could be injected as is, but it is usually prepared in order to optimise the survival of the adipocytes. The aim is to filter or separate the mixture from blood and oil to extract the progenitor cells and functional adipocytes. In 2013, only 5% of the members of the ASPS used additives. The proportion is probably higher today, but the conventional method of preparing harvested fat is to remove the blood, oil and tumescent solution, in one way or another. Common methods of preparation are centrifugation, gravity separation, filtration, washing with saline or Ringer-Acetate and cotton-gauze rolling (the harvested material is poured into a dressing and rolled and massaged gently) (120). There is no consensus regarding which method should be used, however, preparing the fat for injection is favourable, regardless of the method used (116, 124, 125). When comparing non-processed cells with cells subjected to centrifugation and rolling in cotton gauze in a murine study, Canizares et al. found that centrifugation led to a higher proportion of progenitor cells and MSC, while filtering with gauze led to more functional adipocytes, higher post-operative secretion of growth factors, neovascularization and the highest fat graft persistence (126). Moreover, the fat transplant can be enriched before injection. AT-MSC can be isolated and cultivated *ex vivo* before injection. This may increase the survival and quality of the fat transplant (127). A similar approach for enrichment is to use the SVF, which contains a miscellany of progenitor cells. These two enrichment methods are called cell-assisted lipotransfer and have been shown in a meta-analysis to significantly increase the fat survival rate in injection volumes <100ml, but did not reduce the number of procedures needed (128). Both are known to have regenerative abilities. An additive that has been used for enrichment is platelet-rich plasma, but this was not verified to be effective (129).

The last stage, injection, is not as disputed as harvesting and preparation. Most surgeons transfer the fat to small syringes before injection. The fat should be injected slowly in single or multiple layers, depending on the area and indication, using a blunt cannula. It is considered that the diameter of the cannula should be at least 2.5 mm (7, 123) (Figure 4).



. Figure 4. Fat injection with a Coleman cannula.

3.3 INDICATIONS

There are many indications for AFT to the breast. Hypoplasia, asymmetry and tissue deficiency caused by congenital conditions are some of them (124). In breast reconstruction the main indications are asymmetry, tissue defects after breast surgery and RT, deformities, scarring or rippling over implants (124, 130). AFT can be used to fill defects, add volume and loosen scars. Moreover, it has also been shown in clinical studies that radiation-induced fibrosis, radiodermatitis and pain can be ameliorated with AFT (101, 113, 131). Successful whole breast reconstructions with AFT only have been reported, but require multiple procedures (132).

3.4 COMPLICATIONS

AFT is a method with few severe complications. The overall complication rate is generally below 10%. In a systematic review on breast reconstruction, Agha et al. reported a complication rate of 7.3%, fat necrosis being the most common (62% of all complications). 86% were Grade I according to the Clavien-Dindo[2] (133) classification (134). Groen et al. reported a complication rate of 8.4%, with palpable nodules being the most common. Cysts, haematoma, calcification, striae and fat necrosis, in descending order of incidence, were also reported. Less common complications are infections, seroma, pneumothorax and delayed wound healing (135). In a systematic review on healthy breasts, the complication rate for surgical complications was 15.6%, while for complications associated with radiological examination, such as oil cysts, fat necrosis and calcifications that led to biopsies, was 2.2% (136).

3.5 PREVIOUSLY PUBLISHED RESULTS

3.5.1 PROM

There are few comparisons before and after AFT, or comparisons with control groups. However, as mentioned above, it is difficult to define control groups. In a recent meta-analysis, Krastev et al. (100) tried to summarise the disparate values from 34 studies, including one carried out by our group (137). In an attempt to standardise the results from different studies, all the scales were converted into a 10-point scale (continuous data) or dichotomised (categorical data). No information was given on results regarding specific questions such as satisfaction with size. All the results were transformed into an overall degree of satisfaction. The continuous data showed a mean score of 7.4 (95% CI 6.8-8.1) on a 10-point scale, but follow-up time was unknown. The categorical data showed a degree of satisfaction of 94.3% (95% CI 89.9-96.9), for a mean follow-up of 1.9 years. The results from BREAST-Q were analysed separately, giving a general rating of 73.0 (95% CI 67.7-78.4). No analysis was performed of

[2] Clavien-Dindo Grade I: Any deviation from the normal post-operative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgesics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.

pre- and post-AFT scores, and it is therefore not possible to draw any conclusions regarding the impact of AFT on patients' satisfaction with the outcome of AFT alone. Neither did they take, for instance, bias into account in the exclusion criteria. In one of the included studies the surgeon asked two hundred patients at their post-operative visit if they were very satisfied, satisfied, partially satisfied or dissatisfied with the outcome (138). All the patients answered that they were satisfied or very satisfied. This indicates a risk of systematic bias due to a lack of anonymity and a presumed tendency of politeness towards the surgeon.

Until recently, low-quality case series were the only source of information about PRO in the context of breast reconstruction with AFT. In 2017, Bennett et al. (139) published the results of a multicentre cohort study in which the study population consisted of women presenting for breast reconstruction after mastectomy. The cases and controls scored similarly in all four reported domains in BREAST-Q: satisfaction with breasts, physical well-being, psychosocial well-being and sexual well-being, both at baseline and after two years. One limitation of that study was that 18.9% of the controls had undergone AFT previous to the baseline measurement. The authors did not quantify the number of procedures or fat volume. Moreover, the cases and controls differed in vital characteristics such as RT, reconstruction method and whether they had undergone any revisional surgery prior to AFT. It is difficult to draw any conclusions from this study, since the groups are not comparable. In the same year, Cogliandro et al. (140) published the findings of a non-randomised controlled trial in which irradiated and non-irradiated patients who were about to undergo breast reconstruction with permanent implants were enrolled. Two groups were formed: those who had undergone AFT one year after reconstruction, and those who declined further surgery. The groups were compared with BREAST-Q post-operatively. The AFT group scored significantly higher in 12 out of 18 questions addressing satisfaction with breasts. In questions about physical well-being the AFT group reported less pain in the chest area, but no differences in the other parts of the upper body. The results of this study indicate that AFT is a good complement affording breasts that have been reconstructed with implants a more natural appearance, and reducing pain in the region. However, they only measured PRO post-operatively, did not consider multiple testing or tested differences in characteristics between the groups. It is therefore impossible to determine whether there is a causal link.

3.5.2 Radiological imaging after AFT

Few studies on the radiological aspects of AFT have been published during recent years, mainly case reports or case series. A minority included breast cancer patients (141-148). In two different reviews, meta-analysis could not be carried out because of the heterogeneity between studies, and it was therefore difficult to draw any conclusions (134, 149).

Four reviews (134-136, 149) reported that 13-18% of AFT patients had radiological abnormalities, 11.5% needed repeated mammography, and in 2.1-3.7% it was necessary to proceed to biopsy of the abnormality. The most common findings were cysts (11.25-26.7%), fat necrosis (11.3%), micro- and macro- calcifications (1.6-8.7%), suspicious lumps (5.1%) and cancer recurrence (4.2%) (135, 136, 149). It has been reported that larger injected fat volumes may lead to more calcifications and oil cysts (150, 151).

Ultrasound is preferred to identify cysts (135, 149, 152), and mammography to identify calcifications (135). In a study where palpable masses after AFT were examined with ultrasound and compared to histopathological results, it was concluded that ultrasound was a reliable method of distinguishing between benign and malignant lesions after AFT (153). Furthermore, BI-RADS scores seem to be unaffected by AFT (142, 143, 151, 153). However, in one study four of the 39 patients who all hade pre-operative BI-RADS score 1 or 2, had post-operative BI-RADS score 3 or 4 (154).

The potential increase in the need for radiological examinations after AFT has been assessed in a case-control study. The AFT patients had 22% more radiological examinations than the controls, but only mammography was significantly more frequent, probably because of the small sample size (155). Based on the evidence above, AFT to the breast does not seem to have any serious impact on radiological assessment. However, the number of radiological examinations may increase due to increased benign lesions. Since ultrasound is safe and cheap compared to MRI, and a good tool for examining AFT-induced lumps, it may be the first choice when investigating the breast after AFT if a patient presents with a palpable lump.

3.5.3 Effect of AFT on irradiated tissue

3.5.3.1 Clinical effect of AFT on radiation-induced fibrosis and radiodermatitis

There are several reports on a regenerative effect in tissue treated with AFT in both human and animal models (113, 131, 156-158). The LENT-SOMA scale (159) is a tool used to assess tissue damage caused by irradiation[3]. Panettiere et al. reported significantly lower LENT-SOMA scores for pain, telangiectasia, atrophy, breast oedema and fibrosis after AFT to irradiated and reconstructed breasts (131). In a randomised controlled trial on mastectomised and irradiated women, pain was significantly reduced, HRQoL increased and scars were improved according to the observers, but not the patients in the AFT group (101). Rigotti et al. treated 20 patients with LENT-SOMA grade 3 (severe symptoms) or grade 4 (irreversible functional damage) with AFT, and managed to decrease the LENT-SOMA scores significantly. Four of the patients with pre-operative score of 4 decreased to 0, which means cured (113).

3.5.3.2 Biological effect of AFT after irradiation

The biological effects of AFT on radiation-damaged tissue have primarily been explored in animal models, and are still not fully understood (156, 160, 161). Since the main causes of RIF and radiodermatitis seem to be persisting hypoxia and inflammation there are reasons to believe that AT-MSC play a key role in the mechanisms of the positive effects of AFT that we see clinically (113, 156, 162-164). When injected in mice, human AT-MSC secreted vascular endothelial growth factor, especially in a hypoxic environment (165). There are a few animal studies in which the effect of AFT to irradiated skin and fat has been studied by analysing biopsies. Comparison of AT-MSC-enriched AFT-treated and non-AFT-treated irradiated mini pigs, using immunohistochemical analysis of cytokeratin expression showed complete recovery of the epidermis in the ACS pigs. Lymphocyte infiltration, a sign of healing, was detected more than two months earlier than in the controls. An increase in vascularization was seen after the fourth fat transplantation, which was absent in the controls (160). In a murine model, Garza et al. injected human fat subcutaneously four weeks after irradiation. They found dermal

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[3] The LENT-SOMA scale scores pain, oedema, fibrosis/fat necrosis, telangiectasia, arm lymphoedema, retraction/atrophy and ulcer. Grade 1 includes occasional pain, epidermal ulcers and 10-25% retraction. Grade 4 includes useless arm, bone exposure and atrophy of the whole breast.

thickening, higher collagen deposition and hypovascularity, compared to the non-irradiated controls before fat transplantation. Post-transplantation the researchers showed increased vascularity (also reported by Luan et al. (164)), and reduced dermal thickening and collagen content in the irradiated skin. Vascular density did not reach the levels of the controls, and fat retention was higher in the irradiated group (161). The cause of loss of transplant volume seen after AFT (166) may be explained by radiation-induced hypoxia in the tissue (162). Electron microscopy examination of irradiated tissue has shown radiation-induced ischaemic lesions and scleroderma, and a gradual improvement after AFT with normalisation of the microcirculation, leading to better hydration and less fibrosis (113). There is still a lack of detailed knowledge on genetic changes and molecular modifications.

3.6 ONCOLOGICAL SAFETY

The oncological safety of AFT has been debated since the ASPS suggested in 1987 that fat transplantation could make it more difficult to detect early breast cancer. Since 2009, the ASPS has recommended baseline mammography and that surgeons should “exercise caution when considering high-risk patients” based on “little or no systematic empirical evidence”. Since then, very few clinical studies with a high evidence level have been conducted. However, experimental studies examining MSC transplantation *in vivo* and *in vitro* have shown that MSC promote tumour growth and stimulate an inflammatory microclimate favourable for tumour cells in tissues other than breast. On the other hand, other studies have shown that MSC suppress tumour formation and induce apoptosis in skin and blood cancers (167). The evidence is also conflicting in breast tissue. MSC and AFT have been shown to have an immuno-suppressive effect that enables tumour growth and metastasis capacity in murine models and in *in vitro* experiments (168-171). Moreover, it has been suggested that adipose-derived MSC (AT-MSC) promote an inflammatory environment that stimulates tumour proliferation (172). It has also been shown that MSC do not induce neoplasia in healthy adipose tissue (172). These findings have raised concerns that AFT could induce recurrence. No studies have been published on the effect of MSC on tumour cells in irradiated breast tissue. However, in a recent murine experiment, AFT did not stimulate tumour growth and a reduction in the proliferation rate of the tumour cells was observed (173).

In a number of clinical studies and systematic reviews, researchers have tried to rebut the suggestion that AFT could promote or induce recurrence (134, 135, 145, 149, 174). In 2013, it was reported in a systematic review (145), that no inferences could be drawn, even from larger studies, because of incomparable patient groups and diverging results. In 2018, Krastev et al. (175) published a meta-analysis including their own, at that time point, unpublished results (176) and a study assessing recurrence in patients previously reconstructed with DIEP flaps in which it is not specified if the fat was injected in the remaining breast tissue or in the flap. The mean follow-up time was 5.7 years for all AFT patients, and the disease-free interval in matched cohorts was 3.3 years. Meta-analysis of seven matched cohorts showed a difference in the incidence rate of LRR of -0.15% (95% C.I. -0.36-0.07) per year. The lower LRR incidence rate for AFT patients was not statistically significant (p=0.419). A meta-analysis of unmatched cohorts showed a significant (p=0.004) difference in incidence rate of -0.27% (95% C.I. -0.43--0.11) per year, indicating that AFT is protective against LRR. This finding is probably the effect of suboptimal study design, with unmatched controls, demonstrating the importance of robust study design in research. Moreover, pooled data from case series and cohort studies were used to calculate incidence rates. The overall incidence rate was 0.73% (0.56-0.94) per year. Meta-analyses of subgroups showed an incidence rate of 0.79% (0.61-1.01) per year in mastectomy patients, 0.57% (0.23-1.40) per year in BCS patients, 0.83% (0.63-1.09) per year for invasive tumours and 0.45% (0.10-1.89) per year for *in situ* carcinomas. The last sub-analysis is particularly important and interesting since a previous matched case-control study showed a higher risk of recurrence for *in situ* carcinomas (177). The same group conducted a new case-control study on the *in situ* patients and found a 5-year cumulative incidence of local recurrence of 18% in AFT patients, but only 3% in controls (178). These two studies have, for obvious reasons, attracted considerable attention. Recently, the authors published an additional case-control study in which no differences were found in ipsilateral breast cancer recurrence, lymph node metastasis, distant metastasis, contralateral breast cancer, other primary breast cancer or death as a first event. In the discussion, they mention that they found no statistically significant difference between cases and controls in an unpublished long-term follow-up of the patients in the *in situ* study. In a case-control study, it was found that the total fat volume did not affect the risk for recurrence (179). BRCA carriers who have undergone risk-reducing mastectomy and AFT do not seem to have any increased risk of breast carcinomas (180).

In conclusion, concerns have been raised that AFT could promote breast cancer recurrence based on *in vitro* studies. Clinical research has not been able to prove or rebut this suggestion. Current research indicates that AFT does not seem to induce local or distant recurrences. Since randomised controlled trials and cohort studies are not possible, large matched case-control studies are still necessary to establish whether AFT could lead to breast cancer recurrence.

4 AIMS OF THIS RESEARCH

The overall aim of this research was to improve our knowledge of the outcome of autologous fat transplantation when used as a complementary technique in reconstructive plastic surgery in the female breast.

Specific aims of the studies:

- The aims of the first study were to assess the the experiences, and identify possible postoperative complications, of the first group of patients' who had undergone AFT at the Department of Reconstructive Plastic Surgery, Karolinska University Hospital.

- The aim of the second study was to assess patient-reported outcome before and after AFT and to assess the patients' health-related quality of life.

- The aim of the third study was to investigate if AFT could hinder future radiological assessment of the breast and to evaluate changes in the breast after AFT with the two most commonly used radiological methods: mammography and ultrasound.

- The aims of the fourth study were to investigate whether gene expression in adipose breast tissue is altered by irradiation, and whether AFT alters gene expression in irradiated adipose breast tissue.

5 PATIENTS AND METHODS

All the 86 women enrolled in the studies were patients at the Department of Reconstructive Plastic Surgery at Karolinska University Hospital. Some women participated in more than one study (Figure 5). The majority of the patients (73%) had previously been treated with RT.

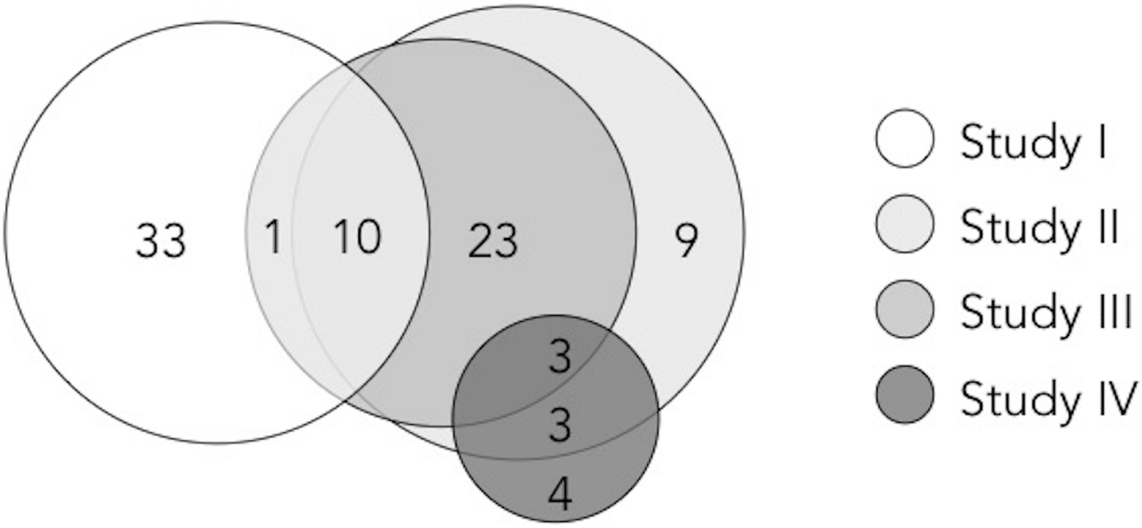

Figure 5. Number of patients from whom data were analysed in each study, and how the patient groups overlap. The total number of patients were 86. Study I: n= 44, Study II: n=48, Study III: n=37 and Study IV: n=10.

5.1 ASSESSMENT OF PATIENTS' EXPERIENCE OF AFT AFTER BREAST RECONSTRUCTION, AND POST-OPERATIVE COMPLICATIONS

Patients who had been treated with AFT after mastectomy and breast reconstruction or BCS at the department between June 2006 and March 2010 were eligible. Women with at least six months follow-up were contacted by post and asked to answer a 12-item questionnaire concerning the outcome. Three of the items contained background questions, and in another three items, the possible responses were yes or no. Nine items were answered on an ordinal scale (much worse, worse, unchanged, better or much better). The items included questions on breast consistency, size, shape, sensibility, skin quality, unevenness, and sequelae at the donor site. In addition to responding to the questions the patients were able to make comments and were asked to describe any problems at the donor site. The senior authors constructed the questionnaire (Appendix 1). It was not psychometrically tested for reliability, validity or sensitivity. If a patient did not respond, a reminder was sent after one to two months. The medical records of the patients were reviewed to identify any complications resulting from AFT.

5.2 ASSESSMENT OF PRO AND HRQOL

Fifty women who had undergone BCS or mastectomy and delayed breast reconstruction, and who presented with poor aesthetic results after surgery and RT, were consecutively enrolled in this prospective case series. These patients were included from October 2008, until June 2013. A flow-diagram of the patients' participation at different time-points is given in Figure 6. The patients underwent 1-4 AFT treatments. Two questionnaires were answered prior to AFT, and six months, one year and two years after final AFT.

A twenty-item study-specific questionnaire (SSQ) was used (Appendix 2). The SSQ has previously been used in studies focusing on other methods of breast reconstruction (181, 182). It was modified for the evaluation of AFT. The original questionnaire was developed by a psychologist, specialised in oncological patients, in association with experienced plastic surgeons (73). Sixteen of the items were rated on a seven-point Likert scale, and considered as continuous in the analysis. The remaining four items were answered on an ordinal scale (not at all, little, pretty much and much). The 16 numerically scored items regards sensitivity, symmetry, aesthetic outcome, consequences of breast cancer treatment (pain and softness) and practical problems. The four ordinal items cover sexuality and difficulties associated with being seen naked. The questionnaire was not psychometrically tested for reliability, validity or sensitivity.

The eight domains of SF-36 were used assess the patients' HRQoL longitudinally and to compare with that of an age matched Swedish female reference population.

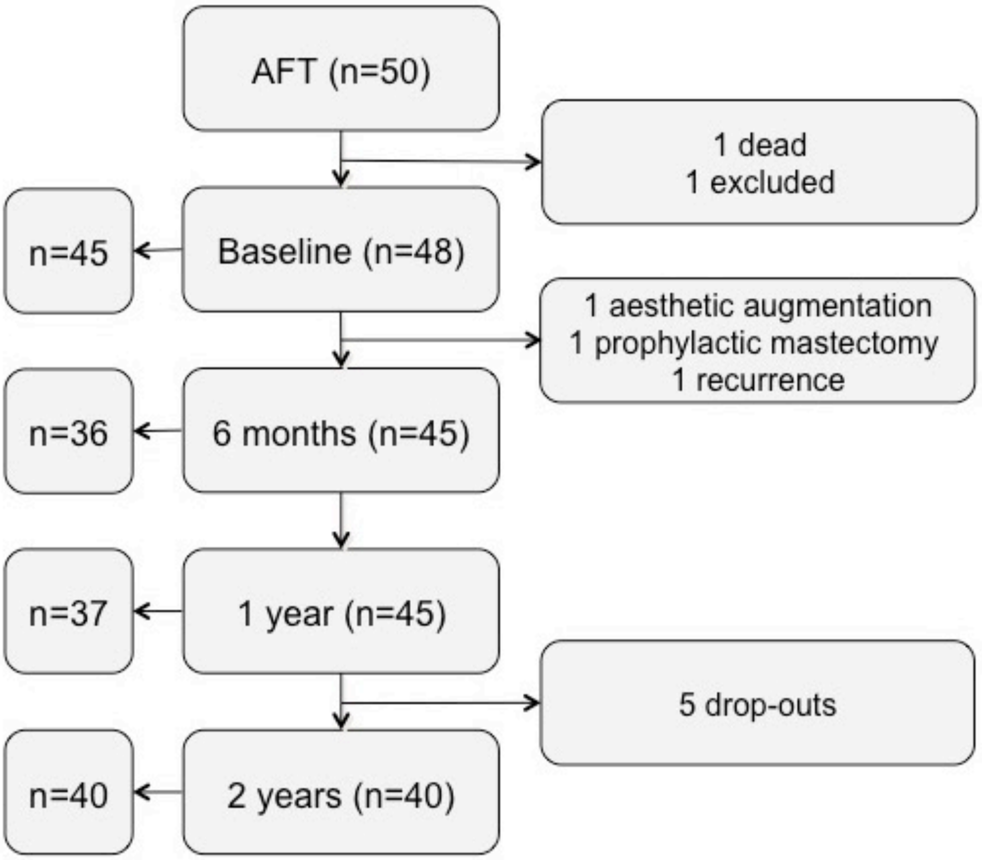

Figure 6. Flow diagram showing patient participation in Study II. Boxes to the left: number of patients who completed the study-specific questionnaire at each time point. Boxes in the middle: number of patients that participated in the study. Boxes to the right: excluded patients and drop-outs.

5.3 ASSESSMENT OF RADIOLOGY

The study population of 37 women (44 breasts) was drawn from the study described in Paper II. Mammography and/or ultrasound were carried out, a maximum of three months before AFT. The patients were re-examined at the planned one-year follow-up. The time point of the intended one-year revisit varied depending on the booking schedule.

Breast radiologists assessed the examinations. The assessment was carried out by manually counting oil cysts and fat necrosis in the preoperative and post-operative mammograms and ultrasound images. The occurrence of calcification and scarring was noted pre- and post-operatively, as well as the progression of pre-existing calcifications and the regression of scarring in the post-operative images. The images were also graded using the BI-RADS score and the pre- and post-operative results were compared. In addition, follow-up-time, the volume of injected fat, and age and time from last surgery to first AFT procedure were compared among the patients with and without findings for all the parameters. For example, age was compared between patients who had oil cysts and those who did not.

5.4 ASSESSMENT OF GENE EXPRESSION ALTERATIONS IN IRRADIATED ADIPOSE TISSUE BEFORE AND AFTER AFT

Thirty irradiated patients were consecutively recruited from the study described in Paper II and another study on AFT patients. Adipose tissue biopsies were taken at the same time from both the irradiated breast and the contralateral non-irradiated breast, the latter providing an internal control. The biopsies were taken before AFT, and about one year after AFT.

5.4.1 Global gene expression profiling

Within our cells the compressed drawing of the entire body is contained in our chromosomes. The chromosomes are DNA molecules carrying our genes. Each gene codes for one, none, several, or a segment of a gene product, most commonly a protein. That process is called gene expression. All genes are not expressed (activated) at the same time and some genes are never expressed, moreover the genes can be expressed to different degrees. The expression of genes is regulated by both intrinsic and external factors and gives us our phenotype. Microarrays offer an efficient analysis of the expression levels of thousands of genes at the same time, called global expression profiling. The main benefit of this technique is that the researcher can have an unbiased approach and does not need to take prior decision on which genes should be examined. The microarray results also provide information on the magnitude of the expression levels. The technique is robust and reproducible and has fast turnaround times. The output from two microarrays (i.e. exposed and non-exposed) can be compared and generate a lists of differentially expressed genes. To identify the relevant biological processes (pathways) that the genes are involved in Gene Set Enrichment Analysis (GSEA) is performed. Gene sets that are involved in specific pathways have been categorised in databases. GSEA identifies enriched pathways in the analysed gene set. In this study RNA extraction, microarray and GSEA (using The Molecular Signatures Database Hallmark Gene Set Collection (183)) were performed at the core facility for Bioinformatics and Expression Analysis at Karolinska Institute.

Two batches of biopsies were analysed with microarray at two different time points (2015 and 2016). The biopsies were divided randomly into the batches. Only patients with all four biopsies with RNA quality and quantity above predetermined thresholds (RIN >4.8 and ≥ 100 ng) were finally analysed. After exclusion only ten patients remained (Figure 7). A batch effect control was made for about 30 000 transcripts in all qualified samples. A small batch effect was seen between the years but, more importantly, not between pre- and post-operative or irradiated and non-irradiated samples meaning that batch effects are less likely to be confounded with the outcomes of interest. The results from the microarray were analysed in two ways: 1. to determine the effect of irradiation on breast adipose tissue, and 2. the effect of AFT on irradiated breast adipose tissue. In the first analysis, gene expression was compared in the irradiated and non-irradiated pre-operative biopsies, and the fold change (FC) calculated. The 3000 most dysregulated transcripts were selected for a GSEA analysis with the Hallmark software to examine pathways affected by irradiation. In the second analysis, the FC for the 3000 genes identified in analysis 1 was calculated for the post-operative biopsies and compared with the pre-operative FC using a paired t-test for each patient and gene to detect genes affected by irradiation and subsequently affected by AFT. Significantly altered genes (high or low pre-operative FC compared to post-operative FC close to 1) were further analysed with Hallmark software to identify pathways associated with an effect caused by AFT.

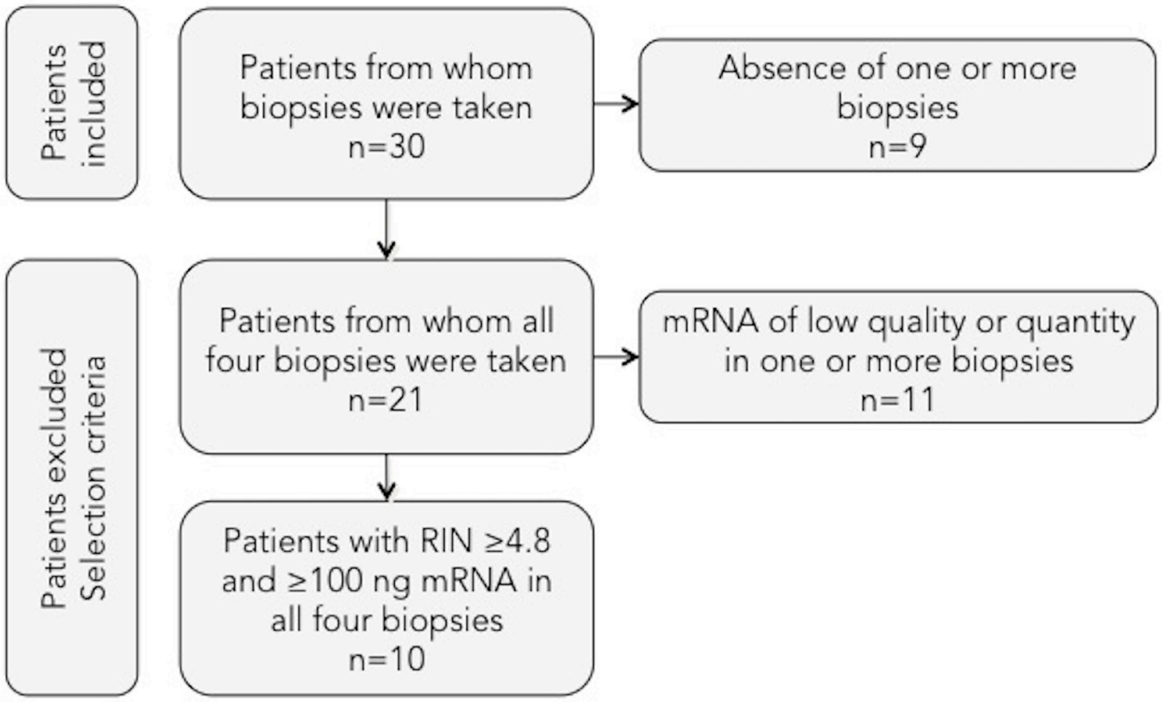

Figure 7. Flow diagram showing the number of patients whose data were included in the final analysis in study IV.

5.4.2 Immunohistochemistry

Immunohistochemistry (IHC) is a method used to visualise cellular components in tissue samples. The most common IHC method is based on antibodies that afford various structures' colour, making them visible in a microscope. The technique was invented already in the 1940s (184) and is widely used in research and as a diagnostic tool in health care. The four biopsies from each of the ten patients were examined with immunohistochemistry (IHC). Macrophages were chosen to evaluate the degree of inflammation in the tissues. CD68 (Cluster of Differentiation 68) is a transmembrane glycoprotein that is highly expressed in tissue macrophages (185). The antibody is routinely used in pathology for diagnostic assays and there are well-established protocols for its use (186-188). CD68 antibody staining was performed to label macrophages. Two independent, blinded co-workers manually quantified stained cells (Figure 8). The ratios of cells per mm^2 in pre- and post-AFT irradiated/non-irradiated samples were compared.

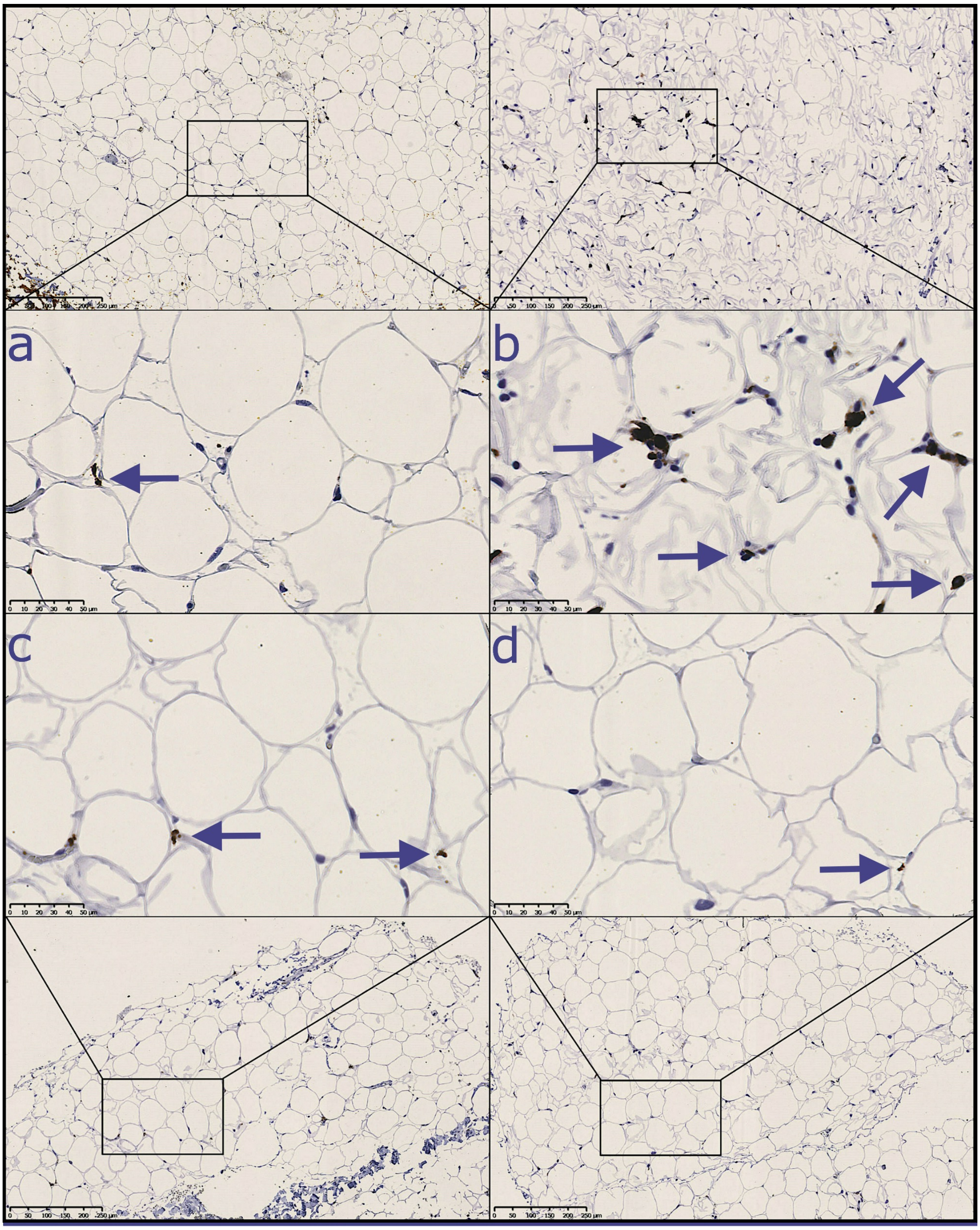

Figure 8. CD68-stained adipose tissue. Arrows indicating stained macrophages and monocytes. a) Non-irradiated adipose tissue b) Irradiated adipose tissue c) Non-irradiated adipose tissue after AFT to the contralateral breast d) Irradiated adipose tissue after AFT

5.5 STATISTICAL METHODS

Study I was a descriptive cross sectional study. No statistical analysis was carried out.

In the second study the numerically scored items (1-4 and 9-20) were evaluated in two ways: firstly, by displaying the results from all patients and time points in mean-line graphs, and secondly the changes in scores between baseline and 12 and 24 months follow-up were analysed using a Mixed effect Model Repeated Measures (MMRM). The outcome variable was the change from baseline and the baseline value was included as a covariate in the model. This model was chosen since it not only takes repeated measures into account, but also missing data. This method is recommended in longitudinal clinical trials rather than imputation or other methods of handling missing data (189). MMRM takes only the patients who completed the questionnaire both before AFT and two years after into account. Two sub-analyses were carried out, one with irradiated patients and one with the patients who had not undergone additional ipsilateral surgery. For the ordinally scored items (5-8) an ordinal trend (OT) was calculated. The OT is the probability that the patient scored a category less positive at baseline than after two years. The items were analysed using a quasi-symmetry model to determine OT. This method is used to calculate trends of flows between categories, and provides an estimate of the probability of the flow of answers from one category to another in an item.

In the third study, differences between the frequencies of the outcomes pre- and post-operatively were tested using McNemar's chi^2 test. The Mann-Whitney test was used to investigate whether age, follow-up time, amount of fat injected or time from last operation to AFT differed between the patients with oil cysts, calcifications/progression of calcifications and regression of scarring and those without. Risk ratios were calculated with 95% confidence intervals.

Since no comparable studies had been published when Study IV was started, it was not possible to carry out any statistical power calculations. A non-paired t-test was used to compare gene expression levels in irradiated and non-irradiated pre-operative biopsies. The FC was calculated in pre-operative biopsies and compared with the FC in the post-operative biopsies. The difference between the FC pre- and post-operatively was then tested with a paired t-test (significance level 5%). Type 1 errors due to multiple testing were taken into account using the false discovery rate (FDR), and q-values <0.05. An FDR is the predicted rate of type 1 errors in a test. No statistical analysis was applied to macrophage density due to the small sample size.

6 RESULTS

6.1 PATIENTS' SATISFACTION WITH AFT AND REPORTED COMPLICATIONS

This study is an evaluation of the first patients that underwent AFT in Karolinska University Hospital. Of the 44 patients invited to take part in this study, one did not answer and could not be located. Her characteristics, complications and volume of injected fat did not differ significantly from those of the other women. Twenty-seven out of the remaining 43 patients had undergone additional surgical procedures. The most frequent procedure was capsulotomy with implant replacement.

Generally, the patients rated the outcome after AFT better than before, apart from sensibility and quality of the skin, for which the majority of patients' scores remained unchanged. Four patients reported adverse effects: two of them reported worse scores for size, shape and irregularities, one patient reported that the size was worse and one of patients that the sensibility had deteriorated. Two patients suffered from pain at the donor site and four had discomforts at the donor site (Table 2). The discomforts were by three patients described as unevenness and indurations and one patient as distension. Only one minor complication was found in the medical records; a local infection which was treated with oral antibiotics.

Table 2 Frequency tables of patients' answers in each category

	Much worse	Worse	Unchanged	Better	Much better
Consistency	0	0	17	22	4
Size	0	3	19	17	3
Shape	0	2	13	21	5
Sensibility	0	1	31	11	0
Skin quality	0	0	35	7	1
Irregularities	0	2	11	24	5

	Yes	No
Pain at the donor site	2	41
Other discomforts at donor site	4	38

6.2 PATIENT-REPORTED OUTCOME AFTER AFT

To be able to measure change over time, the patients were required to answer either the baseline questionnaire or at least two post-operative questionnaires. All questions are given in Table 3. Out of the 50 patients included in this study, two were excluded due to the absence of completed questionnaires. Thirty-eight patients completed the SSQ and 34 the SF-36, both prior to AFT and two years after AFT and were therefore included in the MMRM analyses (Figure 6).

The mean line graphs (Paper 2, Figure 2) show similar patterns, with high scores after six months, followed by a decrease after one year, and an increase after two years. The questions and scores are given in Table 3 and Figure 9. In the MMRM analysis 13 of the 16 items were significantly improved after two years. This was also seen after one year with the exception of softness of the breast. Sub-analyses of the irradiated patients and patients that had not undergone major additional ipsilateral surgery showed similar results after two years. Three out of four items on an ordinal scale had a significant OT. However, the confidence intervals were very wide.

No changes were seen in the eight domains of SF-36 over time. The study group did not largely differ from the reference population, before or after AFT (Paper 2, Figure 4).

Table 3. Questions in SSQ

1	How is the sensation in the nipple area?
2	How is the sensation in the breast skin?
3	How is your ability to feel sexual sensation in the breasts?
4	Do you have pain in the breasts/breast region?
5	Are your breasts of great importance in your sexuality?
6	Do you feel sexually unattractive because of your breasts?
7	Is it hard for you to look at yourself naked because of your breasts?
8	Is it hard for you to show yourself naked to your partner?
9	What do you think about the size of your breasts?
10	Are the breasts of equal size?
11	What do you think of the shape of your breasts?
12	Are the breasts of equal shape?
13	What do you think about the softness of your breasts?
14	Are you satisfied with the appearance of your breasts?
15	What do you think about the appearance of the breast when you wear clothes?
16	What do you think of the breast's appearance when you are wearing swimwear / a bra?
17	What do you think of the breast's appearance when you are naked?
18	What do you think of the scars from previous cancer surgery?
19	Do you have trouble finding a bra that fits your breasts?
20	Do your breasts affect your willingness to swim in public environments (swimming pool, beach, etc.) or participate in gymnastics and similar activities?

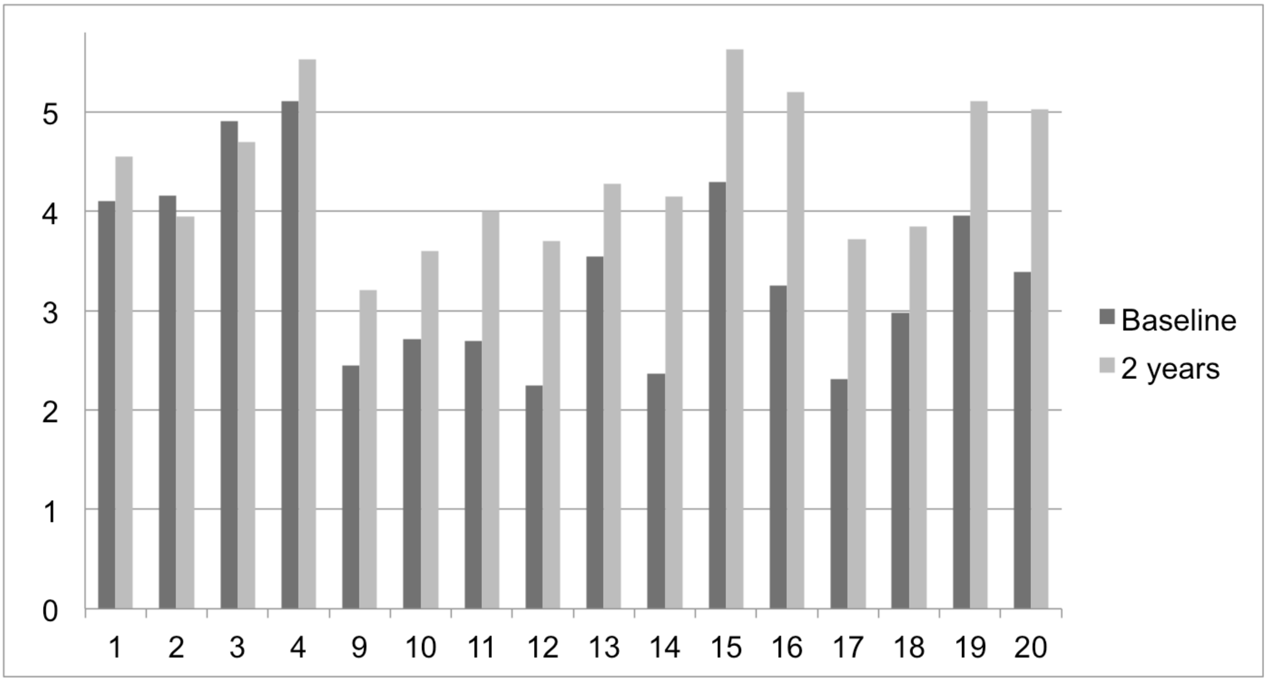

Figure 9. Scores for item 1-4 and 9-20 on a 7-point scale. The questions are given in Table 3.

6.3 EFFECTS OF AFT ON POST-OPERATIVE RADIOLOGICAL ASSESSMENT

All 44 of the breasts examined in Study III had a BI-RADS score of 2 both before and after AFT. Twelve had pre-operative calcifications. Among these, four had an increased number of calcifications post-operatively. Calcifications had developed in five breasts post-operatively. This increase of calcifications after AFT was not statistically significant compared to before AFT. None of the calcifications was clinically relevant and would not have been taken into consideration in clinical practice. Scaring due to previous surgery was seen in all pre-operative mammograms. Reduced scarring was seen in 8 of the 24 post-operative mammograms, but the difference was not significant. Oil cysts were detected with ultrasound in one breast pre-operatively and in 15 post-operatively ($p = 0.0013$). None of the breasts showed any fat necrosis (Paper 3, Table 2).

No differences were found in the follow-up time, volume of fat injected, age at first AFT or time from last operation to first AFT between: a) breasts in which new calcifications and progress in calcification were detected post-operatively and those without, b) breasts with and without reduced scarring, and c) breasts with and without post-operative oil cysts, with the exception that significantly larger fat volumes had been injected into breasts with oil cysts ($p = 0.013$) (Table 4).

Table 4. The results of the analysis in Study III to determine whether follow-up time, injected fat volume, age at surgery or time from last o to first AFT had any impact on the outcome in terms of BI-RADS score, calcifications, scarring, oil cysts or fat necrosis

	BI-RADS	**Calcifications**	**Scaring**	**Oil cysts**	**Necrosis**
Difference between pre- and post-operative examination	No	No	No	Yes	No
Follow-up time	-	No	No	No	No
Injected fat volume	-	No	No	Yes	No
Age at surgery	-	No	No	No	No
Time from last surgery to first AFT	-	No	No	No	No

Six breasts had palpable lumps requiring FNAC. Moreover, the radiologist performed FNAC in three breasts. Oil from one of the lesions was discarded and not further investigated. Cytological examination showed four patients with fat necrosis, two with benign mammary tissue and oil, one with normal adipose tissue and one patient with a cyst.

6.4 EFFECTS OF AFT ON GENE EXPRESSION IN IRRADIATED ADIPOSE TISSUE

In the first analysis, to determine the effect of irradiation on breast adipose tissue, the gene encoding for connective tissue growth factor (CTGF) was among the most dysregulated genes (p=0.0003). It is also present in the EMT pathway, which was the most significantly altered pathway, according to the Hallmark analysis. Interferon gamma (IFNγ) response was the second most altered pathway among the 45 gene sets identified (p-values range from 1.29×10^{-24} to 0.0231 for the 45 pathways). In the second analysis, to determine the effect of AFT on irradiated breast adipose tissue, 575 (19%) of the 3000 genes examined, including the gene for CTGF (p=0.004), showed significantly less variation between the respective sides (p-value range 1.02×10^{-4}-0.0129) after AFT than before. Hallmark analysis of these genes revealed 13 significant pathways. The most altered pathway was IFNγ ($p=1.02\times10^{-5}$) followed by hypoxia ($p=6.38\times10^{-5}$). All pathways affected by AFT were also found among the pathways affected by irradiation (Figure 2, Paper IV). The FDR (expected proportion of falsely rejected null hypotheses) was <0.05 in all analysed transcripts.

The IHC slides from five of the ten patients had acceptable tissue and stain-quality for assessment in all four slides (irradiated and non-irradiated before AFT and irradiated and non-irradiated after AFT to the irradiated side). The cell density ratio (irradiated/non-irradiated before AFT versus irradiated/non-irradiated after AFT) was decreased after AFT in all patients except one.

7 DISCUSSION

Studies on the most important aspects of autologous fat transplantation, namely oncological safety, radiological safety and PRO, are still sparse. Most of the studies have a low evidence level, but point in the same direction with favourable results. It is still not known whether AFT induces recurrence of breast cancer in a certain patient group, or whether AFT makes radiological examinations difficult, possibly leading to missed tumours. Neither do we know the extent to which AFT affects PRO. Moreover, the positive effects of AFT on irradiated tissue have still not been fully explained. These gaps and deficiencies in this research field have also been addressed by Agha et al. who are currently developing a “core outcome set” with the aim of increasing the quality of research in this particular field (190, 191). We do know that the method is popular among both patients and surgeons, and the complication rate is low, with very low morbidity. The research presented in this thesis has improved our knowledge on AFT as a complementary method in breast reconstruction following surgery and radiotherapy for breast cancer.

7.1 STUDY I

At the time of Study I, the effect of AFT on oncological safety and surgical safety was the subject of debate, and no studies had been published on patients’ experience of AFT, apart from one-question surveys. Moreover, reports on occasional adverse effects, such as giant lumps, sepsis and pneumothorax (130, 192-194) made it even more important to evaluate this method, which had recently been implemented at the clinic. It is particularly important to ascertain that surgery performed to increase the patient’s satisfaction, by enhancing the aesthetic outcome, truly benefits the patient and does not lead to harm.

Study I of this thesis presented the first evaluation of a recently implemented complementary surgical procedure and an important first report on patients’ experience. The overall scoring by the patients was positive, indicating that AFT is a good method in terms of PRO for breast reconstruction. Importantly, the complication rate was found to be low. The discomforts reported in the questionnaire were minor and did not need any intervention. As a result of this study, the method gained further acceptance at the clinic, and a more extensive parallel case series study was undertaken to assess PRO. Limitations of Study I are the lack of a control group (either a separate group, or a pre-operative group for comparison with the post-operative group), and the non-validated questionnaire. Moreover, it was a retrospective study, the patient was asked to compare the post-operative result with the pre-operative, which makes it difficult to draw any major inferences from the findings, except that the majority of patients were content with the result and that AFT appears to be a surgically safe method.

7.2 STUDY II

The results in the current study indicate an enhanced appearance of the breast. More importantly AFT appears to benefit the patients in other aspects. The patients report less discomfort with being seen naked, feeling of being more sexually attractive, less pain, improved softness of the breast and willingness to participate in public physical activities. Only four of the 20 items were not significantly improved, sensibility being the most pronounced. This indicates that PRO may be improved several years after AFT. Although the study sample was small, and no control group was available, the findings of this study contribute to the PRO knowledge base. Eleven of the 48 patients underwent additional ipsilateral procedures during the study period. Seven of them were planned nipple surgery to fulfil the breast reconstruction, which was not likely to have any effect on the items studied. Four patients underwent more extensive surgery, which may have affected PRO. One of them was not included in the MMRM analysis because of uncompleted baseline SSQ. The remaining three patients were excluded in a subgroup analysis with the same result as the complete group.

Only two other studies have been published concerning PRO after AFT, both using a more complex instrument than a one-question survey. In a non-randomised trial (140), involving women who had undergone implant-based reconstruction, those receiving AFT reported better fitting of clothes, softness of the breast, symmetry, attractiveness and acceptance of their body, as well as less pain, than the controls (not undergoing AFT). This is in line with the findings of the current study. However, these authors only measured PRO post-operatively, which makes it impossible to know whether the outcome was due to AFT or the implant. In the other study, a large case-control study (139), in which cases and controls had dissimilar characteristics (the controls had more favourable preconditions) and about 20% of the controls had previously undergone AFT, both groups scored similar at baseline and after two years, regarding satisfaction with the breast and sexual, physical and psychosocial well-being. Unfortunately the study did not include comparison of pre- and post-operative scores.

Neither of these studies confirms that AFT has beneficial effects in breast reconstruction, however, they provide no evidence to the contrary.

The HRQoL results of SF-36 did not change largely after AFT. Neither did the results largely differ from the reference population. The small sample size limits the possibility to find small differences between groups and overtime. SF-36 may be too blunt an instrument for investigating changes in HRQoL for this patient group.

7.3 STUDY III

The patients' BI-RADS scores did not change after AFT in this study. In order to answer the question if AFT has any negative effects on future radiological assessment, a case-control study with the same approach, with pre- and post-operative radiological examinations would have been more appropriate. Long-term follow-up, with the assessment of yearly mammograms would therefore be necessary to investigate whether local recurrences have the same appearance in AFT-treated breasts as in non-treated breasts. No cases of fat necrosis were identified with radiology, but four were diagnosed with FNAC. It is possible that the experience of fat necrosis differs between specialists, and its evaluation is, in many ways, subjective. Fat necrosis is one of the most commonly reported complications of AFT and a common management is therefore desirable.

The findings of this study showed a probable causality between large amounts of injected fat and the formation of oil cysts. This is important knowledge for surgeons, and valuable information for reducing anxiety in concerned patients. The inconsistency of the results may be due to variations in surgical techniques and differences in radiologists' stringency in reporting findings. The main disadvantage of AFT is the necessity for the investigation of benign masses and radiological findings induced by AFT, which would otherwise not be required.

7.4 STUDY IV

To the best of the authors' knowledge, no other studies have been performed involving global gene expression profiling of irradiated human breast adipose tissue. In the present study, gene expression was compared before and after AFT. The prospective setting, with the patients as their own controls, provides the opportunity to examine biological mechanisms related to AFT in irradiated human tissue.

CTGF is known to be correlated to fibrotic cascades caused by irradiation in vascular and lung tissue (51, 195, 196). CTGF has also been shown to activate EMT in mice (197). Moreover, EMT is known to be activated by irradiation in other tissues and contributes significantly to the development of RIF (42, 45, 51, 198). In the present study, CTGF was one of the most up-regulated genes in the irradiated pre-operative biopsy samples, and EMT the most affected pathway. Irradiation has been shown to cause hypovascularity and vasculopathy, as well as inducing hypoxia, which also plays a major role in RIF, causes perivascular matrix deposition, and induces extracellular matrix synthesis (47-52, 113, 161, 164). AFT has been shown to gradually improve and normalise microcirculation with higher hydration and less fibrosis (113). The current findings, of highly dysregulated CTGF gene expression and altered EMT and Hypoxia pathways in human adipose tissue correlate with existing knowledge from other types of irradiated tissue, and therefore provide a relevant contribution to the literature. Moreover, EMT and hypoxia were two of the 13 pathways that were significantly reversed after AFT, which implies that AFT has a biological healing effect on RIF. The IFNγ response pathway is associated with inflammatory responses to ionizing radiation, and inflammatory cytokines play an essential role in the development of fibrosis (50, 199, 200). In this current study IFNγ was highly dysregulated before AFT showing that radiation-induced reaction is continuing years after RT. Dysregulated gene expression patterns for several genes in the IFNγ pathway was nearly normalised in the irradiated breast after AFT. An increase in macrophage influx after RT and a reduction after AFT have been reported previously in both humans and mice (113, 201). Moreover, the macrophage density was higher in irradiated tissue before AFT than after AFT. This implies that inflammatory responses are activated a long time after RT in adipose tissue. The effects of irradiation were seen up to 14 years after radiotherapy in the present study.

The experiments in this study were conducted on RNA level, and therefore describe only gene expression and not what happens on the protein level. However, the findings correlate with available data and support observations in animal models.

7.5 LIMITATIONS OF THE STUDIES

No obvious control group is available when assessing PRO in relation to AFT as a complement to BCS or breast reconstruction. A case-control study would suffer from reduced precision since the controls would not have any indication for AFT, and would thus be a different population. A cohort study would not be practical since the group of AFT patients is rather small, although the number is increasing. However, such a cohort would have to be very large. Moreover, a cohort study would suffer from the same problem with controls as in a case-control study. The only other possible study design would be a randomised controlled trial in which half of the patients with indications for AFT would not be offered this alternative. This would not be ethically acceptable given that the hypothesis is that AFT is superior to no AFT. Thus, a case-series may be the only possible option.

The two study-specific questionnaires used in Studies I and II were not validated, and therefore constitute limitations. However, when the two studies started, no validated questionnaires relevant for breast reconstruction were available in Swedish, therefore the only choice was to develop our own questionnaires.

All the studies suffer from small sample size, resulting in low statistical power. During the inclusion period, the number of eligible patients was small, and thus only limited numbers of patients could be recruited. The situation today has improved. The number of patients in Study IV is in the normal range for this kind of experimental gene expression study. The internal controls gave the study higher statistical power.

A large number of variables were tested in Studies II and III. There is thus a risk of type I errors, false-positives, due to multiple testing, especially in Study II where most of the null hypotheses were rejected. In Study IV the FDR was used to make allowances for multiple testing and was <0.05 in all analysed pathways.

7.6 INTERNAL VALIDITY

The study-specific questionnaires used in Studies I and II may be a source of systematic errors since they were not validated. The reliability over time should not be affected, as the measurements would be equally incorrect both before and after AFT. However, the accuracy could be affected. The Swedish reference population for SF-36 was generated in 1991-92, and it is possible that the HRQoL has changed since then. Comparison of the present study group with the Swedish reference population may therefore be less correct, but the comparison between time points will still be valid.

7.6.1 Bias

7.6.1.1 Selection bias

There is thought to be little selection bias in the present studies as the patients reflect the patient group with indications for AFT. One could off cause speculate that challenging patients were not offered AFT at all. Patients not invited to participate in the studies did not receive any equivalent treatment, and were therefore not even eligible for inclusion. On the other hand, the medical assessment of whether the patient would benefit from AFT or not is subjective, and surgeons who suggest surgical procedures in public health care are influenced by the patient's wish or willingness to undergo further surgery. At the time of inclusion, this was a new method for the surgeons, and the indications for AFT may have changed, intentionally or unintentionally, with increased experience of the surgeons. If this is the case, the patients in Studies I and II may perhaps not be representative of all patients undergoing AFT today.

7.6.1.2 Information bias

There may be a problem with recall bias in Study I since the patients were asked to assess the outcome compared to conditions before AFT.

7.6.1.3 *Missing data*

A lack of completed questionnaires at different time points could induce bias. Attempts were made to avoid this in Study II by using MMRM.

7.6.2 Confounding

In Studies I-III, surgery other than AFT performed during the study period could be a confounding factor. This was avoided by excluding all patients who underwent additional surgery in a sub-analysis. In Study IV a batch effect could have occurred since microarray was carried out at two time points. Batch effect controls showed insignificant effect of the time-related aspect. No batch effect was found in the non-irradiated control biopsies over time (from before to after AFT), but differed from the irradiated breast biopsies both pre and post AFT.

7.6.3 Effect modification

Time from breast cancer surgery and RT could affect the patients' answers in Studies I and II. Time heals, in Study II the range in time from RT was one to 22 years with a median of about 3.5. It is possible that the patients with one and 22 years history answered differently just because of time. In Study IV, the time from RT to AFT could affect the outcome positively or negatively. The effects of irradiation may be more profound after a short period and then diminish over time. However, it has been seen that these effects are sustained over years in many patients. The gene expression in chronically radiation-damaged tissue may also change over time. No adjustment was made for time from RT to AFT.

7.7 EXTERNAL VALIDITY

Studies I and II were conducted in an urban setting in Sweden, a wealthy country with one of the world's highest degrees of equality. It could be speculated that this may have an effect on how important the appearance of her breasts is to a woman. Satisfaction with, and perception of, body image differ between cultures (202). There is also a difference in the rate of aesthetic breast augmentation between Western countries. In 2016, the incidence of breast augmentation was about 0.03% in Sweden, whereas in France, Italy and the US the incidence was two to three times higher (203, 204). This difference could reflect the prevailing societal attitude to the importance of the appearance of breasts. AFT and breast reconstruction are available through the national health service in Sweden. It is therefore possible that patients undergoing this form of surgery in countries where breast reconstruction is not generally available, or health insurance is required, may differ socioeconomically from the study population in this research. In Study III, radiologists assessed the increased amount of calcification in the breast, the amount of scarring and the reduction in scarring subjectively. There is thus reason to believe that radiologists may assess differently. The findings of Study IV could probably be generalised to other populations. There is no reason to believe that radiation-induced changes in gene expression in other populations would diverge from that of Swedish women. If differences in gene expression in adipose tissue between populations exist, it is more likely that the effect of the injected healthy adipocytes on gene expression would diverge. The internal controls increase the generalizability.

7.8 PRECISION

Random errors could be present as a result of the small study groups. Furthermore, a large amount of data from the questionnaires and patients' medical records were entered manually into spreadsheets for analysis, and some data could have been entered erroneously. In Study IV, random errors could have occurred during RNA preparation, microarray analysis or staining procedures. Experienced analysts at the core facility for Bioinformatics and Expression Analysis and at the Department of Clinical Pathology at Karolinska University Hospital performed all the laboratory work, which should have decreased the risk of such errors.

8 CONCLUSIONS

The main conclusions from the studies on AFT for corrective surgery after breast conserving surgery and radiotherapy or breast reconstruction with or without previous radiotherapy, presented in this thesis, are given below.

- A majority of women who have undergone AFT, report improvements postoperatively. AFT results in few and minor postoperative complications.

- AFT enhances the patient-reported aesthetic outcome. The women become more comfortable with being seen naked, consider themself more sexually attractive and are more willing to participate in public physical activities. AFT may also reduce pain and make the breast tissue softer, alleviating the adverse effects of breast cancer surgery and RT. The study groups HRQoL was comparable to that of a Swedish reference population.

- AFT does not appear to negatively affect radiological assessment of the breast when performed by experienced radiologists. AFT can cause oil cysts and larger volume of injected fat appears to increase the risk of developing oil cysts.

- Irradiation appears to have a persisting effect on gene expression in breast adipose tissue. AFT could conceivably reverse irradiation-induced gene expression alterations several years after radiotherapy. Among the detected genes, biological pathways associated with fibrosis, inflammation and hypoxia apperes to be the most enriched.

9 FUTURE PERSPECTIVES

Given that it is still uncertain to what degree the patients benefit from repeated AFT surgery with limited fat volumes, the aesthetic result alone may not necessarily be the main indication for AFT in public health care in Sweden. The indications should probably be broadened to include other aspects. About 15-25% of patients treated surgically for breast cancer suffer from long-lasting pain in the area (205). Patients suffering from pain resulting from surgery or the adverse effects of RT could be a future patient group. Studies have shown that AFT can reduce pain in the area. It has also been shown in clinical studies that chronic inflammation and fibrosis resulting from irradiation can be reversed with AFT, which was also found in this work. Moreover, performing AFT prior to breast reconstruction with implants after RT may increase the chances of a complication-free procedure (206), or make it possible to use this reconstruction method in more patients than today.

Total breast reconstructions with AFT have been carried out in other countries (132, 207). Mastectomised women, previously treated with or without adjuvant RT, have undergone repeated AFT procedures to achieve the desired breast volume. An American plastic surgeon has invented the BRAVA device, a plastic dome that is placed over the chest to expand the tissue externally with continuous suction before breast reconstruction with AFT (115). Total breast reconstruction with AFT may be one method of the future to reconstruct autologous breasts with minimal surgery, at least for smaller breasts. Larger volumes of injected fat could also be used to achieve symmetry as an alternative or complement to contralateral breast reduction.

Sweden has a well-established system of registries, for example, national quality registries, cancer registries and the Swedish cause of death registry. Most of these registries are of high quality and have a high coverage rate. A registry-based case-control study in collaboration with the Clinical Epidemiology Core Facility at the Karolinska Institute would increase our knowledge on oncological safety. All breast cancer patients who have undergone AFT at Karolinska University Hospital, and, if possible, also at other university hospitals in Sweden, could be matched with breast cancer patients who have not undergone AFT by age, breast cancer stage, treatment and time since the completion of treatment. The outcomes studied could be progression-free survival and death from breast cancer.

It would also be interesting to conduct a randomised controlled trial in collaboration with specialists in pain medicine, including patients who suffer from pain in the area of previous breast surgery or breast reconstruction. The patients would be allocated either to continuing their current treatment/strategy or to receiving best-known medical treatment or AFT. A similar study has been carried out in Denmark with good results, showing decreased pain after AFT but these findings remain to be confirmed (101).

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11 SAMMANFATTNING PÅ SVENSKA

Autolog fettransplantation (AFT) är en metod för att förbättra ett brösts utseende efter bröstcancerkirurgi, strålbehandling eller bröstrekonstruktion. AFT kan bland annat användas för att jämna ut oregelbundenheter, justera volymsunderskott och mjuka upp hård ärrvävnad. Fett skördas genom fettsugning på till exempel buken. Det prepareras och injiceras sedan i fina kanaler i det område som ska behandlas. I Sverige är idag huvudsyftet med AFT förbättring av patientens livskvalitet, men trots detta är forskningen om patienters erfarenheter av metoden begränsad. Vidare råder det osäkerhet om huruvida AFT försvårar bedömningen av radiologiska undersökningar. På 1980-talet skrev The American Society of Plastic Surgeons att användandet av AFT skulle kunna dölja cancer samt leda till falskt positiva radiologiska fynd och onödiga undersökningar. Idag avråder de inte från metoden på grund av bristande underlag men uppmanar till ytterligare studier i ämnet. Utöver estetisk förbättring, kan AFT användas för att behandla strålskador som uppstår i hud och underliggande vävnader efter strålbehandling och som kan kvarstå i många år. Effekten av AFT på strålskador, så som kronisk inflammation och fibros, är väl beskriven i kliniska studier men de biologiska processerna är fortfarande okända. Målet för detta arbete var att med bakgrund av ovanstående undersöka patientrapporterat utfall, radiologi samt genuttryck i fett efter AFT. Avhandlingen består av fyra studier med syfte att öka kunskapen om patientrapporterat utfall, radiologi samt genexpression i strålad bröstvävnad före och efter AFT.

Den första studien genomfördes för att utvärdera en för kliniken då ny metod. Syftet var att undersöka de första patienternas erfarenhet och identifiera eventuella komplikationer. Syftet med den andra studien var att undersöka patientrapporterat utfall genom att jämföra pre- och postoperativa självskattningsformulär samt bedöma patienternas livskvalitet. I den tredje studien var syftet att studera vilka effekter AFT haft på den radiologiska bedömningen av mammografi och ultraljud. Syftet med den fjärde studien var att undersöka huruvida genuttryck i bröstfettvävnad förändras efter strålbehandling och vidare om AFT i sin tur förändrar genuttryck i den strålade bröstvävnaden.

Studie I är en retrospektiv studie som utvärderar 43 patienters upplevelse av AFT med ett studiespecifikt frågeformulär efter operationen. Komplikationer från 44 patienter registrerades. En majoritet av patienterna rapporterade förbättringar eter AFT. En patient hade fått en lokal infektion, vilken behandlades med peroral antibiotika.

Studie II är en prospektiv fallserie i vilken patientrapporterat utfall undersöktes bland 48 patienter med ett studiespecifikt frågeformulär och 36-item Short Form Health Survey (SF-36), ett skattningsformulär för hälsorelaterad livskvalitet, före och två år efter AFT. I sexton av 20 frågor svarade patienterna mer positivt efter två år. Förbättring hade skett bland annat av bröststorlek, bröstform, utseende på bröstet, mjukhet, ärr, smärta, vilja att visa sig naken för partner och se sig själv naken, känsla av att känna sig sexuellt oattraktiv, möjlighet att hitta passande BH samt vilja delta i fysiska aktiviteter i offentliga miljöer. Ingen skillnad kunde ses i skattningen av känsel i bröstet. Resultatet för hälsorelaterad livskvalitet ändrades inte efter AFT. Patienternas skattning låg även i samma nivå som en referenspopulation av svenska kvinnor.

Studie III utvärderar resultat av mammografi och ultraljud hos 44 patienter före och ett år efter AFT. Det visades ingen skillnad i den övergripande bedömningen, samtliga patienter hade BI-RADS[4] score 2 (godartat utseende) både före och efter AFT. AFT ökade inte signifikant mängden förkalkningar eller fettnekroser men minskade inte heller ärrvävnaden. Antalet bröst med oljecystor ökade signifikant och det sågs också att bröst med oljecystor hade fått större volymer fett injicerat.

Studie IV studerar genuttryck i bröstfettvävnad. Fettbiopsier togs från tio kvinnors strålbehandlade bröst samt det andra icke-strålbehandlade bröstet före AFT. Detta upprepades ett år efter att endast det strålade bröstet behandlats med AFT. Genuttrycket (de gener i DNAt som transkriberas till RNA och som oftast har proteiner som slutprodukt) undersöktes med hjälp av microarray för kvantifiering samt Hallmark gene set enrichment analysis som är en mjukvara för identifiering av de signaleringsvägar (biologiska processer) som de undersökta generna ingår i. Bland de 3000 gener med mest förändrat uttryck i en jämförelse av strålad och icke-strålad fettvävnad, fann man fyrtiofem signaleringsvägar som var påverkade av strålning. Efter AFT, i jämförelsen av strålad och AFT-behandlad fettvävnad med icke-strålad fettvävnad, var 19 % (575) av de 3000 generna inte längre signifikant förändrade och

[4] Breast Imaging Reporting and Data System

således påverkade av AFT-behandlingen. Bland de 575 generna identifierades 13 signaleringsvägar som alla även återfinns bland de 45 initiala. De viktigaste signaleringsvägarna var de för interferon gamma respons som reglerar inflammationsreaktioner, hypoxi (syrebrist) vilket uppstår vid strålning och bidrar till de skadliga effekterna av denna samt epitelial mesenkymal transition som är en viktig komponent vid fibrosbildning vilket i sin tur leder till hypoxi. Med hjälp av immunhistokemi sågs ett ökat antal makrofager i strålad vävnad. Detta kunde inte ses efter AFT. Ett signifikant samband kunde dock ej styrkas.

Slutsatserna är att autolog fettransplantation är en metod som tolereras väl av patienterna, vilka upplever förbättring i flera avseenden utöver förbättrad estetik. Metoden försvårar inte den radiologiska bedömningen men ökar antal oljecystor vid injektion med större volymer. Strålbehandling ändrar uttrycket i bröstfettvävnad för gener som är involverade i signaleringsvägar för inflammation, hypoxi och fibros. AFT verkar kunna motverka dysregleringen av dessa signaleringsvägar.

12 REFERENCES

1. Mazzola RF, Cantarella G, Torretta S, Sbarbati A, Lazzari L, Pignataro L. Autologous fat injection to face and neck: from soft tissue augmentation to regenerative medicine. Acta otorhinolaryngologica Italica : organo ufficiale della Societa italiana di otorinolaringologia e chirurgia cervico-facciale. 2011;31(2):59-69.
2. Illouz YG. Body contouring by lipolysis: a 5-year experience with over 3000 cases. Plastic and Reconstructive Surgery. 1983;72(5):591-7.
3. Bircoll M. Autologous fat transplantation. Plastic and Reconstructive Surgery. 1987;79(3):492-3.
4. Bircoll M, Novack BH. Autologous fat transplantation employing liposuction techniques. Annals of plastic surgery. 1987;18(4):327-9.
5. Coleman SR. Facial recontouring with lipostructure. Clin Plast Surg. 1997;24(2):347-67.
6. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. Aesthetic Plast Surg. 1995;19(5):421-5.
7. Coleman SR. Structural fat grafting. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 1998;18(5):386, 8.
8. Procedures AA-HCoN. Report on autologous fat transplantation. September 30, 1987. Plastic surgical nursing : official journal of the American Society of Plastic and Reconstructive Surgical Nurses. 1987;7(4):140-1.
9. Halsted WS. I. The Results of Operations for the Cure of Cancer of the Breast Performed at the Johns Hopkins Hospital from June, 1889, to January, 1894. Annals of surgery. 1894;20(5):497-555.
10. Madden JL, Kandalaft S, Bourque RA. Modified radical mastectomy. Annals of surgery. 1972;175(5):624-34.
11. Group SSBC. NATIONELLA RIKTLINJER FÖR BEHANDLING AV BRÖSTCANCER 2018. 2018.
12. (Socialstyrelsen) TNBoHaW. Socialstyrelsens Cancerregister 2017 [Available from: https://www.socialstyrelsen.se/statistik/statistikdatabas/cancer.
13. Engholm G FJ, Christensen N, Hansen HL, Hertzum-Larsen R, Johannesen TB, Kejs AMT, Khan S, Ólafsdóttir E, Petersen T, Schmidt LKH, Virtanen A, Storm HH. . NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries, Version 8.0 (20.12.2017). . Association of the Nordic Cancer Registries Danish Cancer Society Available from http://wwwancrnu, accessed on day/month/year 2017.
14. Autier P, Boniol M, La Vecchia C, Vatten L, Gavin A, Hery C, et al. Disparities in breast cancer mortality trends between 30 European countries: retrospective trend analysis of WHO mortality database. BMJ (Clinical research ed). 2010;341:c3620.
15. Allemani C, Minicozzi P, Berrino F, Bastiaannet E, Gavin A, Galceran J, et al. Predictions of survival up to 10 years after diagnosis for European women with breast cancer in 2000-2002. International journal of cancer. 2013;132(10):2404-12.
16. (NKBC) Nkfb. Årsrapport 2017 [Internet] 2017 [Available from: http://statistik.incanet.se/brostcancer/.
17. Socialstyrelsen. Bröstcancer, screening med mammografi 2018 [Available from: http://www.socialstyrelsen.se/riktlinjer/nationellascreeningprogram/brostcancer-screeningmedmammog.

18. The benefits and harms of breast cancer screening: an independent review. Lancet. 2012;380(9855):1778-86.
19. Gøtzsche PC, Jørgensen KJ. Screening for breast cancer with mammography. Cochrane Database of Systematic Reviews. 2013(6).
20. Ma L, Fishell E, Wright B, Hanna W, Allan S, Boyd NF. Case-control study of factors associated with failure to detect breast cancer by mammography. Journal of the National Cancer Institute. 1992;84(10):781-5.
21. Pettersson A, Graff RE, Ursin G, Santos Silva ID, McCormack V, Baglietto L, et al. Mammographic density phenotypes and risk of breast cancer: a meta-analysis. Journal of the National Cancer Institute. 2014;106(5).
22. C.J. D'Orsi EBM, D.M. Ikeda, et al. Breast imaging reporting and data system: ACR BI-RADS – breast imaging atlas. American College of Radiology, Reston (VA) 2003.
23. Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378(9793):771-84.
24. (EBCTCG) EBCTCG. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365(9472):1687-717.
25. Cuzick J, Ambroisine L, Davidson N, Jakesz R, Kaufmann M, Regan M, et al. Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials. Lancet. 2007;369(9574):1711-23.
26. Adam H, Bygdeson M, de Boniface J. The oncological safety of nipple-sparing mastectomy - a Swedish matched cohort study. European Journal of Surgical Oncology. 2014;40(10):1209-15.
27. Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. The New England journal of medicine. 2002;347(16):1233-41.
28. Curran D, van Dongen JP, Aaronson NK, Kiebert G, Fentiman IS, Mignolet F, et al. Quality of life of early-stage breast cancer patients treated with radical mastectomy or breast-conserving procedures: results of EORTC Trial 10801. The European Organization for Research and Treatment of Cancer (EORTC), Breast Cancer Co-operative Group (BCCG). European journal of cancer (Oxford, England : 1990). 1998;34(3):307-14.
29. Iwuchukwu OC, Harvey JR, Dordea M, Critchley AC, Drew PJ. The role of oncoplastic therapeutic mammoplasty in breast cancer surgery--a review. Surgical oncology. 2012;21(2):133-41.
30. Clough KB, Benyahi D, Nos C, Charles C, Sarfati I. Oncoplastic surgery: pushing the limits of breast-conserving surgery. The breast journal. 2015;21(2):140-6.
31. Bramhall RJ, Lee J, Concepcion M, Westbroek D, Huf S, Mohammed K, et al. Central round block repair of large breast resection defects: oncologic and aesthetic outcomes. Gland surgery. 2017;6(6):689-97.
32. Clough KB, Ihrai T, Oden S, Kaufman G, Massey E, Nos C. Oncoplastic surgery for breast cancer based on tumour location and a quadrant-per-quadrant atlas. The British journal of surgery. 2012;99(10):1389-95.

33. Anderson BO, Masetti R, Silverstein MJ. Oncoplastic approaches to partial mastectomy: an overview of volume-displacement techniques. The Lancet Oncology. 2005;6(3):145-57.
34. Ogawa T, Hanamura N, Yamashita M, Ito M, Kimura H, Nakamura T, et al. Abdominal advancement flap as oncoplastic breast conservation: report of seven cases and their cosmetic results. Journal of Breast Cancer. 2013;16(2):236-43.
35. Arver B, Isaksson K, Atterhem H, Baan A, Bergkvist L, Brandberg Y, et al. Bilateral prophylactic mastectomy in Swedish women at high risk of breast cancer: a national survey. Annals of surgery. 2011;253(6):1147-54.
36. Hartmann LC, Lindor NM. The Role of Risk-Reducing Surgery in Hereditary Breast and Ovarian Cancer. The New England journal of medicine. 2016;374(5):454-68.
37. Ahmed M, Purushotham AD, Horgan K, Klaase JM, Douek M. Meta-analysis of superficial versus deep injection of radioactive tracer and blue dye for lymphatic mapping and detection of sentinel lymph nodes in breast cancer. The British journal of surgery. 2015;102(3):169-81.
38. Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet. 2011;378(9804):1707-16.
39. de Boniface J, Frisell J, Bergkvist L, Andersson Y. Breast-conserving surgery followed by whole-breast irradiation offers survival benefits over mastectomy without irradiation. The British journal of surgery. 2018.
40. Sjövall K, Strömbeck G, Löfgren A, Bendahl PO, Gunnars B. Adjuvant radiotherapy of women with breast cancer – Information, support and side-effects. European Journal of Oncology Nursing. 2010;14(2):147-53.
41. Leventhal J, Young MR. Radiation Dermatitis: Recognition, Prevention, and Management. Oncology (Williston Park, NY). 2017;31(12):885-7, 94-9.
42. Straub JM, New J, Hamilton CD, Lominska C, Shnayder Y, Thomas SM. Radiation-induced fibrosis: mechanisms and implications for therapy. Journal of cancer research and clinical oncology. 2015;141(11):1985-94.
43. Bentzen SM, Thames HD, Overgaard M. Latent-time estimation for late cutaneous and subcutaneous radiation reactions in a single-follow-up clinical study. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 1989;15(3):267-74.
44. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European organization for research and treatment of cancer (EORTC). International Journal of Radiation Oncology*Biology*Physics. 1995;31(5):1341-6.
45. Weigel C, Schmezer P, Plass C, Popanda O. Epigenetics in radiation-induced fibrosis. Oncogene. 2015;34(17):2145-55.
46. Poglio S, Galvani S, Bour S, Andre M, Prunet-Marcassus B, Penicaud L, et al. Adipose tissue sensitivity to radiation exposure. The American journal of pathology. 2009;174(1):44-53.
47. Choi SH, Hong ZY, Nam JK, Lee HJ, Jang J, Yoo RJ, et al. A Hypoxia-Induced Vascular Endothelial-to-Mesenchymal Transition in Development of Radiation-Induced Pulmonary Fibrosis. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(16):3716-26.
48. Halle M, Gabrielsen A, Paulsson-Berne G, Gahm C, Agardh HE, Farnebo F, et al. Sustained inflammation due to nuclear factor-kappa B activation in irradiated human arteries. Journal of the American College of Cardiology. 2010;55(12):1227-36.

49. Christersdottir Bjorklund T, Reilly SJ, Gahm C, Bottazzi B, Mantovani A, Tornvall P, et al. Increased long-term expression of pentraxin 3 in irradiated human arteries and veins compared to internal controls from free tissue transfers. Journal of translational medicine. 2013;11:223.
50. Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. Radiation research. 2012;178(6):505-23.
51. Kumar R, Griffin M, Adigbli G, Kalavrezos N, Butler PE. Lipotransfer for radiation-induced skin fibrosis. The British journal of surgery. 2016;103(8):950-61.
52. Hoving S, Heeneman S, Gijbels MJ, Te Poele JA, Visser N, Cleutjens J, et al. Irradiation induces different inflammatory and thrombotic responses in carotid arteries of wildtype C57BL/6J and atherosclerosis-prone ApoE(-/-) mice. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology. 2012;105(3):365-70.
53. Stone HB, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. The Lancet Oncology. 2003;4(9):529-36.
54. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer letters. 2012;327(1-2):48-60.
55. Schaue D, Micewicz ED, Ratikan JA, Xie MW, Cheng G, McBride WH. Radiation and inflammation. Seminars in radiation oncology. 2015;25(1):4-10.
56. Halle M, Bodin I, Tornvall P, Wickman M, Farnebo F, Arnander C. Timing of radiotherapy in head and neck free flap reconstruction--a study of postoperative complications. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2009;62(7):889-95.
57. Krueger EA, Wilkins EG, Strawderman M, Cederna P, Goldfarb S, Vicini FA, et al. Complications and patient satisfaction following expander/implant breast reconstruction with and without radiotherapy. International journal of radiation oncology, biology, physics. 2001;49(3):713-21.
58. Lee BT, T AA, Colakoglu S, Curtis MS, Yueh JH, K EA, et al. Postmastectomy radiation therapy and breast reconstruction: an analysis of complications and patient satisfaction. Annals of plastic surgery. 2010;64(5):679-83.
59. Eriksson M, Anveden L, Celebioglu F, Dahlberg K, Meldahl I, Lagergren J, et al. Radiotherapy in implant-based immediate breast reconstruction: risk factors, surgical outcomes, and patient-reported outcome measures in a large Swedish multicenter cohort. Breast cancer research and treatment. 2013;142(3):591-601.
60. Liljegren G, Holmberg L, Westman G. The cosmetic outcome in early breast cancer treated with sector resection with or without radiotherapy. Uppsala-Orebro Breast Cancer Study Group. European journal of cancer (Oxford, England : 1990). 1993;29a(15):2083-9.
61. Benediktsson K, Perbeck L. Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: five years of monitoring of a prospective trial. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2006;59(1):27-34.
62. Devulapalli C, Bello RJ, Moin E, Alsobrooks J, Fallas PB, Ohkuma R, et al. The Effect of Radiation on Quality of Life throughout the Breast Reconstruction Process: A Prospective, Longitudinal Pilot Study of 200 Patients with Long-Term Follow-Up. Plastic and Reconstructive Surgery. 2018;141(3):579-89.
63. Schaverien MV, Macmillan RD, McCulley SJ. Is immediate autologous breast reconstruction with postoperative radiotherapy good practice?: a systematic review of the literature. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2013;66(12):1637-51.

64. Longo B, Laporta R, Sorotos M, Pagnoni M, Gentilucci M, Santanelli di Pompeo F. Total breast reconstruction using autologous fat grafting following nipple-sparing mastectomy in irradiated and non-irradiated patients. Aesthetic Plast Surg. 2014;38(6):1101-8.
65. Wickman M. Breast reconstruction--past achievements, current status and future goals. Scandinavian journal of plastic and reconstructive surgery and hand surgery / Nordisk plastikkirurgisk forening [and] Nordisk klubb for handkirurgi. 1995;29(2):81-100.
66. Cronin T D GFJ. Augmentation mammaplasty; A new natural feel prosthesis. . Transactions Third International Congress, Plastic and Reconstructive Surgeons; Excerpta Medica, Amsterdam1964. p. 41-9.
67. Lewis JR, Jr. THE AUGMENTATION MAMMAPLASTY, WITH SPECIAL REFERENCE TO ALLOPLASTIC MATERIALS. Plastic and Reconstructive Surgery. 1965;35:51-9.
68. Becker H. Breast reconstruction using an inflatable breast implant with detachable reservoir. Plastic and Reconstructive Surgery. 1984;73(4):678-83.
69. Tasoulis MK, Iqbal FM, Cawthorn S, MacNeill F, Vidya R. Subcutaneous implant breast reconstruction: Time to reconsider? European Journal of Surgical Oncology. 2017;43(9):1636-46.
70. Gruber RP, Kahn RA, Lash H, Maser MR, Apfelberg DB, Laub DR. Breast reconstruction following mastectomy: a comparison of submuscular and subcutaneous techniques. Plastic and Reconstructive Surgery. 1981;67(3):312-7.
71. Wilson AR, Marotti L, Bianchi S, Biganzoli L, Claassen S, Decker T, et al. The requirements of a specialist Breast Centre. European journal of cancer (Oxford, England : 1990). 2013;49(17):3579-87.
72. Ehinger A, Lindman H, Löfgren L, Petersson L-M, Sandelin K. Årsrapport från Nationella Bröstcancerregistret 2015.; 2016.
73. Brandberg Y, Malm M, Blomqvist L. A prospective and randomized study, "SVEA," comparing effects of three methods for delayed breast reconstruction on quality of life, patient-defined problem areas of life, and cosmetic result. Plastic and Reconstructive Surgery. 2000;105(1):66-74; discussion 5-6.
74. Eltahir Y, Werners LL, Dreise MM, van Emmichoven IA, Jansen L, Werker PM, et al. Quality-of-life outcomes between mastectomy alone and breast reconstruction: comparison of patient-reported BREAST-Q and other health-related quality-of-life measures. Plastic and Reconstructive Surgery. 2013;132(2):201e-9e.
75. Spear SL, Murphy DK, Slicton A, Walker PS. Inamed silicone breast implant core study results at 6 years. Plastic and Reconstructive Surgery. 2007;120(7 Suppl 1):8S-16S; discussion 7S-8S.
76. Cunningham B. The Mentor Core Study on Silicone MemoryGel Breast Implants. Plastic and Reconstructive Surgery. 2007;120(7 Suppl 1):19S-29S; discussion 30S-2S.
77. Bertozzi N, Pesce M, Santi P, Raposio E. Tissue expansion for breast reconstruction: Methods and techniques. Annals of Medicine and Surgery. 2017;21:34-44.
78. Lindegren A, Halle M, Docherty Skogh AC, Edsander-Nord A. Postmastectomy breast reconstruction in the irradiated breast: a comparative study of DIEP and latissimus dorsi flap outcome. Plastic and Reconstructive Surgery. 2012;130(1):10-8.
79. Olivari N. The latissimus flap. British journal of plastic surgery. 1976;29(2):126-8.

80. Johns N, Fairbairn N, Trail M, Ewing A, Yong L, Raine C, et al. Autologous breast reconstruction using the immediately lipofilled extended latissimus dorsi flap. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2018;71(2):201-8.
81. Sinna R, Delay E, Garson S, Delaporte T, Toussoun G. Breast fat grafting (lipomodelling) after extended latissimus dorsi flap breast reconstruction: A preliminary report of 200 consecutive cases. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2010;63(11):1769-77.
82. Holmstrom H, Lossing C. The lateral thoracodorsal flap in breast reconstruction. Plastic and Reconstructive Surgery. 1986;77(6):933-43.
83. Holmstrom H. The free abdominoplasty flap and its use in breast reconstruction. An experimental study and clinical case report. Scandinavian journal of plastic and reconstructive surgery. 1979;13(3):423-27.
84. Allen RJ, Treece P. Deep inferior epigastric perforator flap for breast reconstruction. Annals of plastic surgery. 1994;32(1):32-8.
85. Nelson EC, Eftimovska E, Lind C, Hager A, Wasson JH, Lindblad S. Patient reported outcome measures in practice. BMJ : British Medical Journal. 2015;350.
86. Sullivan M, Karlsson J, Taft C, Ware JE. SF-36 Hälsoenkät: Svensk Manual och Tolkningsguide, 2:a upplagan (Swedish Manual and Interpretation Guide, 2nd Edition). Gothenburg: Sahlgrenska University Hospital2004.
87. Sullivan M, Karlsson J, Ware JE, Jr. The Swedish SF-36 Health Survey--I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. Social science & medicine (1982). 1995;41(10):1349-58.
88. Cano SJ, Klassen AF, Scott AM, Cordeiro PG, Pusic AL. The BREAST-Q: further validation in independent clinical samples. Plastic and Reconstructive Surgery. 2012;129(2):293-302.
89. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. Plastic and Reconstructive Surgery. 2009;124(2):345-53.
90. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. Health economics. 1993;2(3):217-27.
91. Nationella Kvalitetsregister, Sveriges Kommuner och Landsting 2017 [http://kvalitetsregister.se/drivaregister/patientmedverkan/patientrapporteradematt.1988.html].
92. Orwelius L, Nilsson M, Nilsson E, Wenemark M, Walfridsson U, Lundstrom M, et al. The Swedish RAND-36 Health Survey - reliability and responsiveness assessed in patient populations using Svensson's method for paired ordinal data. Journal of patient-reported outcomes. 2017;2(1):4.
93. Winters ZE, Afzal M, Rutherford C, Holzner B, Rumpold G, da Costa Vieira RA, et al. International validation of the European Organisation for Research and Treatment of Cancer QLQ-BRECON23 quality-of-life questionnaire for women undergoing breast reconstruction. The British journal of surgery. 2018;105(3):209-22.
94. Carifio J, Perla R. Resolving the 50-year debate around using and misusing Likert scales. Medical education. 2008;42(12):1150-2.
95. Sullivan GM, Artino AR, Jr. Analyzing and interpreting data from likert-type scales. Journal of graduate medical education. 2013;5(4):541-2.
96. Groen JW, Piatkowski AA, Sawor JH, Wilschut JA, Khouri RK, van der Hulst R, et al. Autologous Fat Transfer After Augmentation and Reconstruction of the Female Breast: An International, Cross-Sectional Photo-Comparison Study Among Different Physician and Laymen Study Groups. Surgical innovation. 2018:1553350618798435.

97. Wachter T, Edlinger M, Foerg C, Djedovic G, Mayerl C, Kinzl J, et al. Differences between patients and medical professionals in the evaluation of aesthetic outcome following breast reconstruction with implants. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2014;67(8):1111-7.
98. Kim MS, Sbalchiero JC, Reece GP, Miller MJ, Beahm EK, Markey MK. Assessment of breast aesthetics. Plastic and Reconstructive Surgery. 2008;121(4):186e-94e.
99. Beesley H, Ullmer H, Holcombe C, Salmon P. How patients evaluate breast reconstruction after mastectomy, and why their evaluation often differs from that of their clinicians. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2012;65(8):1064-71.
100. Krastev TK, Alshaikh GAH, Hommes J, Piatkowski A, van der Hulst R. Efficacy of autologous fat transfer for the correction of contour deformities in the breast: A systematic review and meta-analysis. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2018.
101. Juhl AA, Karlsson P, Damsgaard TE. Fat grafting for alleviating persistent pain after breast cancer treatment: A randomized controlled trial. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2016;69(9):1192-202.
102. Cardoso JS, Cardoso MJ. Towards an intelligent medical system for the aesthetic evaluation of breast cancer conservative treatment. Artificial intelligence in medicine. 2007;40(2):115-26.
103. Penn J. Breast reduction. British journal of plastic surgery. 1955;7(4):357-71.
104. Smith DJ, Jr., Palin WE, Jr., Katch VL, Bennett JE. Breast volume and anthropomorphic measurements: normal values. Plastic and Reconstructive Surgery. 1986;78(3):331-5.
105. Westreich M. Anthropomorphic breast measurement: protocol and results in 50 women with aesthetically perfect breasts and clinical application. Plastic and Reconstructive Surgery. 1997;100(2):468-79.
106. Chae MP, Rozen WM, Spychal RT, Hunter-Smith DJ. Breast volumetric analysis for aesthetic planning in breast reconstruction: a literature review of techniques. Gland surgery. 2016;5(2):212-26.
107. O'Connell RL, Stevens RJ, Harris PA, Rusby JE. Review of three-dimensional (3D) surface imaging for oncoplastic, reconstructive and aesthetic breast surgery. Breast (Edinburgh, Scotland). 2015;24(4):331-42.
108. Howes BH, Watson DI, Fosh B, Yip JM, Kleinig P, Dean NR. Magnetic Resonance Imaging Versus 3-Dimensional Laser Scanning for Breast Volume Assessment After Breast Reconstruction. Annals of plastic surgery. 2017;78(4):455-9.
109. Maione L, Vinci V, Caviggioli F, Klinger F, Banzatti B, Catania B, et al. Autologous Fat Graft in Postmastectomy Pain Syndrome Following Breast Conservative Surgery and Radiotherapy. Aesthetic Plast Surg. 2014.
110. Coleman SR. Hand rejuvenation with structural fat grafting. Plastic and Reconstructive Surgery. 2002;110(7):1731-44; discussion 45-7.
111. Guisantes E, Fontdevila J, Rodriguez G. Autologous fat grafting for correction of unaesthetic scars. Annals of plastic surgery. 2012;69(5):550-4.
112. Sultan SM, Barr JS, Butala P, Davidson EH, Weinstein AL, Knobel D, et al. Fat grafting accelerates revascularisation and decreases fibrosis following thermal injury. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2012;65(2):219-27.
113. Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process

mediated by adipose-derived adult stem cells. Plastic and Reconstructive Surgery. 2007;119(5):1409-22; discussion 23-4.
114. Bar-Meir ED, Yueh JH, Tobias AM, Lee BT. Autologous fat grafting: a technique for stabilization of the microvascular pedicle in DIEP flap reconstruction. Microsurgery. 2008;28(7):495-8.
115. Khouri RK, Jr., Khouri RK. Current Clinical Applications of Fat Grafting. Plastic and Reconstructive Surgery. 2017;140(3):466e-86e.
116. Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The Current State of Fat Grafting: A Review of Harvesting, Processing, and Injection Techniques. Plastic and Reconstructive Surgery. 2015;136(4):897-912.
117. Varghese J, Griffin M, Mosahebi A, Butler P. Systematic review of patient factors affecting adipose stem cell viability and function: implications for regenerative therapy. Stem cell research & therapy. 2017;8(1):45.
118. Padoin AV, Braga-Silva J, Martins P, Rezende K, Rezende AR, Grechi B, et al. Sources of processed lipoaspirate cells: influence of donor site on cell concentration. Plastic and Reconstructive Surgery. 2008;122(2):614-8.
119. Jurgens WJ, Oedayrajsingh-Varma MJ, Helder MN, Zandiehdoulabi B, Schouten TE, Kuik DJ, et al. Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. Cell and tissue research. 2008;332(3):415-26.
120. Kling RE, Mehrara BJ, Pusic AL, Young VL, Hume KM, Crotty CA, et al. Trends in autologous fat grafting to the breast: a national survey of the american society of plastic surgeons. Plastic and Reconstructive Surgery. 2013;132(1):35-46.
121. Klein JA. The tumescent technique. Anesthesia and modified liposuction technique. Dermatologic clinics. 1990;8(3):425-37.
122. Fournier PF, Otteni FM. Lipodissection in body sculpturing: the dry procedure. Plastic and Reconstructive Surgery. 1983;72(5):598-609.
123. Sinno S, Wilson S, Brownstone N, Levine SM. Current Thoughts on Fat Grafting: Using the Evidence to Determine Fact or Fiction. Plastic and Reconstructive Surgery. 2016;137(3):818-24.
124. Gutowski KA, Force AFGT. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. Plastic and Reconstructive Surgery. 2009;124(1):272-80.
125. Fisher C, Grahovac TL, Schafer ME, Shippert RD, Marra KG, Rubin JP. Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation. Plastic and Reconstructive Surgery. 2013;132(2):351-61.
126. Canizares O, Jr., Thomson JE, Allen RJ, Jr., Davidson EH, Tutela JP, Saadeh PB, et al. The Effect of Processing Technique on Fat Graft Survival. Plastic and Reconstructive Surgery. 2017;140(5):933-43.
127. Trojahn Kolle SF, Oliveri RS, Glovinski PV, Elberg JJ, Fischer-Nielsen A, Drzewiecki KT. Importance of mesenchymal stem cells in autologous fat grafting: a systematic review of existing studies. Journal of plastic surgery and hand surgery. 2012;46(2):59-68.
128. Laloze J, Varin A, Gilhodes J, Bertheuil N, Grolleau JL, Brie J, et al. Cell-assisted lipotransfer: Friend or foe in fat grafting? Systematic review and meta-analysis. Journal of tissue engineering and regenerative medicine. 2018;12(2):e1237-e50.
129. Rigotti G, Charles-de-Sa L, Gontijo-de-Amorim NF, Takiya CM, Amable PR, Borojevic R, et al. Expanded Stem Cells, Stromal-Vascular Fraction, and Platelet-Rich Plasma Enriched Fat: Comparing Results of Different Facial Rejuvenation Approaches

in a Clinical Trial. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 2016;36(3):261-70.
130. Delay E, Garson S, Tousson G, Sinna R. Fat Injection to the Breast: Technique, Results, and Indications Based on 880 Procedures Over 10 Years. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 2009;29(5):360-76.
131. Panettiere P, Marchetti L, Accorsi D. The serial free fat transfer in irradiated prosthetic breast reconstructions. Aesthetic Plast Surg. 2009;33(5):695-700.
132. Hoppe DL, Ueberreiter K, Surlemont Y, Peltoniemi H, Stabile M, Kauhanen S. Breast reconstruction de novo by water-jet assisted autologous fat grafting--a retrospective study. German medical science : GMS e-journal. 2013;11:Doc17.
133. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Annals of surgery. 2004;240(2):205-13.
134. Agha RA, Fowler AJ, Herlin C, Goodacre TE, Orgill DP. Use of autologous fat grafting for breast reconstruction: a systematic review with meta-analysis of oncological outcomes. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2015;68(2):143-61.
135. Groen JW, Negenborn VL, Twisk DJ, Rizopoulos D, Ket JC, Smit JM, et al. Autologous fat grafting in onco-plastic breast reconstruction: A systematic review on oncological and radiological safety, complications, volume retention and patient/surgeon satisfaction. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2016;69(6):742-64.
136. Largo RD, Tchang LA, Mele V, Scherberich A, Harder Y, Wettstein R, et al. Efficacy, safety and complications of autologous fat grafting to healthy breast tissue: a systematic review. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2014;67(4):437-48.
137. Schultz I, Lindegren A, Wickman M. Improved shape and consistency after lipofilling of the breast: patients' evaluation of the outcome. Journal of plastic surgery and hand surgery. 2012;46(2):85-90.
138. Sinna R, Delay E, Garson S, Delaporte T, Toussoun G. Breast fat grafting (lipomodelling) after extended latissimus dorsi flap breast reconstruction: a preliminary report of 200 consecutive cases. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2010;63(11):1769-77.
139. Bennett KG, Qi J, Kim HM, Hamill JB, Wilkins EG, Mehrara BJ, et al. Association of Fat Grafting With Patient-Reported Outcomes in Postmastectomy Breast Reconstruction. JAMA surgery. 2017;152(10):944-50.
140. Cogliandro A, Barone M, Tenna S, Morelli Coppola M, Persichetti P. The Role of Lipofilling After Breast Reconstruction: Evaluation of Outcomes and Patient Satisfaction with BREAST-Q. Aesthetic Plast Surg. 2017;41(6):1325-31.
141. Brenelli F, Rietjens M, De Lorenzi F, Pinto-Neto A, Rossetto F, Martella S, et al. Oncological safety of autologous fat grafting after breast conservative treatment: a prospective evaluation. The breast journal. 2014;20(2):159-65.
142. Carvajal J, Patino JH. Mammographic findings after breast augmentation with autologous fat injection. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 2008;28(2):153-62.
143. Costantini M, Cipriani A, Belli P, Bufi E, Fubelli R, Visconti G, et al. Radiological findings in mammary autologous fat injections: A multi-technique evaluation. Clinical Radiology. 2013;68(1):27-33.

144. Kim HY, Jung BK, Lew DH, Lee DW. Autologous Fat Graft in the Reconstructed Breast: Fat Absorption Rate and Safety based on Sonographic Identification. Archives of plastic surgery. 2014;41(6):740-7.
145. Krastev TK, Jonasse Y, Kon M. Oncological safety of autologous lipoaspirate grafting in breast cancer patients: a systematic review. Annals of surgical oncology. 2013;20(1):111-9.
146. Rietjens M, De Lorenzi F, Rossetto F, Brenelli F, Manconi A, Martella S, et al. Safety of fat grafting in secondary breast reconstruction after cancer. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2011;64(4):477-83.
147. Rubin JP, Coon D, Zuley M, Toy J, Asano Y, Kurita M, et al. Mammographic changes after fat transfer to the breast compared with changes after breast reduction: a blinded study. Plastic and Reconstructive Surgery. 2012;129(5):1029-38.
148. Wang CF, Zhou Z, Yan YJ, Zhao DM, Chen F, Qiao Q. Clinical analyses of clustered microcalcifications after autologous fat injection for breast augmentation. Plastic and Reconstructive Surgery. 2011;127(4):1669-73.
149. Claro F, Jr., Figueiredo JC, Zampar AG, Pinto-Neto AM. Applicability and safety of autologous fat for reconstruction of the breast. The British journal of surgery. 2012;99(6):768-80.
150. Wang H, Jiang Y, Meng H, Zhu Q, Dai Q, Qi K. Sonographic identification of complications of cosmetic augmentation with autologous fat obtained by liposuction. Annals of plastic surgery. 2010;64(4):385-9.
151. Lindegren A, Chantereau MW, Bygdeson M, Azavedo E, Schultz I. Autologous Fat Transplantation to the Reconstructed Breast Does not Hinder Assessment of Mammography and Ultrasound: A Cohort Study. World journal of surgery. 2016;40(5):1104-11.
152. Fiaschetti V, Pistolese CA, Fornari M, Liberto V, Cama V, Gentile P, et al. Magnetic resonance imaging and ultrasound evaluation after breast autologous fat grafting combined with platelet-rich plasma. Plastic and Reconstructive Surgery. 2013;132(4):498e-509e.
153. Parikh RP, Doren EL, Mooney B, Sun WV, Laronga C, Smith PD. Differentiating fat necrosis from recurrent malignancy in fat-grafted breasts: an imaging classification system to guide management. Plastic and Reconstructive Surgery. 2012;130(4):761-72.
154. Juhl AA, Redsted S, Engberg Damsgaard T. Autologous fat grafting after breast conserving surgery: Breast imaging changes and patient-reported outcome. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2018.
155. Pinell-White XA, Etra J, Newell M, Tuscano D, Shin K, Losken A. Radiographic Implications of Fat Grafting to the Reconstructed Breast. The breast journal. 2015;21(5):520-5.
156. Mojallal A, Lequeux C, Shipkov C, Breton P, Foyatier JL, Braye F, et al. Improvement of skin quality after fat grafting: clinical observation and an animal study. Plastic and Reconstructive Surgery. 2009;124(3):765-74.
157. Costantino A, Fioramonti P, Ciotti M, Onesti MG. Lipofilling in skin affected by radiodermatitis: clinical and ultrasound aspects. Case report. Il Giornale di chirurgia. 2012;33(5):186-90.
158. Salgarello M, Visconti G, Barone-Adesi L. Fat grafting and breast reconstruction with implant: another option for irradiated breast cancer patients. Plastic and Reconstructive Surgery. 2012;129(2):317-29.
159. LENT SOMA scales for all anatomic sites. International journal of radiation oncology, biology, physics. 1995;31(5):1049-91.

160. Forcheron F, Agay D, Scherthan H, Riccobono D, Herodin F, Meineke V, et al. Autologous adipocyte derived stem cells favour healing in a minipig model of cutaneous radiation syndrome. PloS one. 2012;7(2):e31694.
161. Garza RM, Paik KJ, Chung MT, Duscher D, Gurtner GC, Longaker MT, et al. Studies in fat grafting: Part III. Fat grafting irradiated tissue--improved skin quality and decreased fat graft retention. Plastic and Reconstructive Surgery. 2014;134(2):249-57.
162. Pallua N, Pulsfort AK, Suschek C, Wolter TP. Content of the growth factors bFGF, IGF-1, VEGF, and PDGF-BB in freshly harvested lipoaspirate after centrifugation and incubation. Plastic and Reconstructive Surgery. 2009;123(3):826-33.
163. Shukla L, Morrison WA, Shayan R. Adipose-derived stem cells in radiotherapy injury: a new frontier. Frontiers in surgery. 2015;2:1.
164. Luan A, Duscher D, Whittam AJ, Paik KJ, Zielins ER, Brett EA, et al. Cell-Assisted Lipotransfer Improves Volume Retention in Irradiated Recipient Sites and Rescues Radiation-Induced Skin Changes. Stem cells (Dayton, Ohio). 2016;34(3):668-73.
165. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109(10):1292-8.
166. Hivernaud V, Lefourn B, Guicheux J, Weiss P, Festy F, Girard AC, et al. Autologous Fat Grafting in the Breast: Critical Points and Technique Improvements. Aesthetic Plast Surg. 2015;39(4):547-61.
167. Nicolay NH, Lopez Perez R, Debus J, Huber PE. Mesenchymal stem cells - A new hope for radiotherapy-induced tissue damage? Cancer letters. 2015;366(2):133-40.
168. Ljujic B, Milovanovic M, Volarevic V, Murray B, Bugarski D, Przyborski S, et al. Human mesenchymal stem cells creating an immunosuppressive environment and promote breast cancer in mice. Scientific reports. 2013;3:2298.
169. Martin-Padura I, Gregato G, Marighetti P, Mancuso P, Calleri A, Corsini C, et al. The white adipose tissue used in lipotransfer procedures is a rich reservoir of CD34+ progenitors able to promote cancer progression. Cancer research. 2012;72(1):325-34.
170. Kamat P, Schweizer R, Kaenel P, Salemi S, Calcagni M, Giovanoli P, et al. Human Adipose-Derived Mesenchymal Stromal Cells May Promote Breast Cancer Progression and Metastatic Spread. Plastic and Reconstructive Surgery. 2015;136(1):76-84.
171. Massa M, Gasparini S, Baldelli I, Scarabelli L, Santi P, Quarto R, et al. Interaction Between Breast Cancer Cells and Adipose Tissue Cells Derived from Fat Grafting. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 2016;36(3):358-63.
172. Eterno V, Zambelli A, Pavesi L, Villani L, Zanini V, Petrolo G, et al. Adipose-derived Mesenchymal Stem Cells (ASCs) may favour breast cancer recurrence via HGF/c-Met signaling. Oncotarget. 2014;5(3):613-33.
173. Tsuji W, Valentin JE, Marra KG, Donnenberg AD, Donnenberg VS, Rubin JP. An Animal Model of Local Breast Cancer Recurrence in the Setting of Autologous Fat Grafting for Breast Reconstruction. Stem cells translational medicine. 2018;7(1):125-34.
174. Waked K, Colle J, Doornaert M, Cocquyt V, Blondeel P. Systematic review: The oncological safety of adipose fat transfer after breast cancer surgery. Breast (Edinburgh, Scotland). 2017;31:128-36.

175. Krastev TK, Schop SJ, Hommes J, Piatkowski AA, Heuts EM, van der Hulst R. Meta-analysis of the oncological safety of autologous fat transfer after breast cancer. The British journal of surgery. 2018.
176. Krastev T, van Turnhout A, Vriens E, Smits L, van der Hulst R. Long-term Follow-up of Autologous Fat Transfer vs Conventional Breast Reconstruction and Association With Cancer Relapse in Patients With Breast Cancer. JAMA surgery. 2018.
177. Petit JY, Botteri E, Lohsiriwat V, Rietjens M, De Lorenzi F, Garusi C, et al. Locoregional recurrence risk after lipofilling in breast cancer patients. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012;23(3):582-8.
178. Petit JY, Rietjens M, Botteri E, Rotmensz N, Bertolini F, Curigliano G, et al. Evaluation of fat grafting safety in patients with intraepithelial neoplasia: a matched-cohort study. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2013;24(6):1479-84.
179. Silva-Vergara C, Fontdevila J, Weshahy O, Yuste M, Descarrega J, Grande L. Breast Cancer Recurrence Is not Increased With Lipofilling Reconstruction: A Case-Controlled Study. Annals of plastic surgery. 2017;79(3):243-8.
180. Kronowitz SJ, Mandujano CC, Liu J, Kuerer HM, Smith B, Garvey P, et al. Lipofilling of the Breast Does Not Increase the Risk of Recurrence of Breast Cancer: A Matched Controlled Study. Plastic and Reconstructive Surgery. 2016;137(2):385-93.
181. Edsander-Nord A, Brandberg Y, Wickman M. Quality of life, patients' satisfaction, and aesthetic outcome after pedicled or free TRAM flap breast surgery. Plastic and Reconstructive Surgery. 2001;107(5):1142-53; discussion 54-5.
182. Gahm J, Jurell G, Edsander-Nord A, Wickman M. Patient satisfaction with aesthetic outcome after bilateral prophylactic mastectomy and immediate reconstruction with implants. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2010;63(2):332-8.
183. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell systems. 2015;1(6):417-25.
184. Coons AH, Creech HJ, Jones RN. Immunological Properties of an Antibody Containing a Fluorescent Group. Proceedings of the Society for Experimental Biology and Medicine. 1941;47(2):200-2.
185. Holness CL, Simmons DL. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. Blood. 1993;81(6):1607-13.
186. Cordell JL FB, Flenghi L, Jones DB, Pileri S, Radzun HJ, et al. M15. CD68 cluster workshop report. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 925-7.
187. Falini B, Flenghi L, Pileri S, Gambacorta M, Bigerna B, Durkop H, et al. PG-M1: a new monoclonal antibody directed against a fixative-resistant epitope on the macrophage-restricted form of the CD68 molecule. The American journal of pathology. 1993;142(5):1359-72.
188. Falini B FL, Pileri S, Stein H, Dürkop H, Pasqualucci L, et al. M15.1. PG-M1 a new mAb directed against a fixative-resistant, macrophage-restricted epitope of the CD68 molecule. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 928-30.

189. Mallinckrod† CH, Lane PW, Schnell D, Peng Y, Mancuso JP. Recommendations for the Primary Analysis of Continuous Endpoints in Longitudinal Clinical Trials. Drug Information Journal. 2008;42(4):303-19.
190. Agha RA, Fowler AJ, Pidgeon TE, Wellstead G, Orgill DP. The Need for Core Outcome Reporting in Autologous Fat Grafting for Breast Reconstruction. Annals of plastic surgery. 2016;77(5):506-12.
191. Agha RA, Pidgeon TE, Borrelli MR, Dowlut N, Orkar TK, Ahmed M, et al. Validated Outcomes in the Grafting of Autologous Fat to the Breast: The VOGUE Study. Development of a Core Outcome Set for Research and Audit. Plastic and Reconstructive Surgery. 2018;141(5):633e-8e.
192. Hyakusoku H, Ogawa R, Ono S, Ishii N, Hirakawa K. Complications after autologous fat injection to the breast. Plastic and Reconstructive Surgery. 2009;123(1):360-70; discussion 71-2.
193. Talbot SG, Parrett BM, Yaremchuk MJ. Sepsis after autologous fat grafting. Plastic and Reconstructive Surgery. 2010;126(4):162e-4e.
194. Valdatta L, Thione A, Buoro M, Tuinder S. A case of life-threatening sepsis after breast augmentation by fat injection. Aesthetic Plast Surg. 2001;25(5):347-9.
195. Bickelhaupt S, Erbel C, Timke C, Wirkner U, Dadrich M, Flechsig P, et al. Effects of CTGF Blockade on Attenuation and Reversal of Radiation-Induced Pulmonary Fibrosis. Journal of the National Cancer Institute. 2017;109(8).
196. Khan AA, Paget JT, McLaughlin M, Kyula JN, Wilkinson MJ, Pencavel T, et al. Genetically modified lentiviruses that preserve microvascular function protect against late radiation damage in normal tissues. Science translational medicine. 2018;10(425).
197. Sonnylal S, Xu S, Jones H, Tam A, Sreeram VR, Ponticos M, et al. Connective tissue growth factor causes EMT-like cell fate changes in vivo and in vitro. Journal of cell science. 2013;126(Pt 10):2164-75.
198. Kawamoto A, Yokoe T, Tanaka K, Saigusa S, Toiyama Y, Yasuda H, et al. Radiation induces epithelial-mesenchymal transition in colorectal cancer cells. Oncology reports. 2012;27(1):51-7.
199. Di Maggio FM, Minafra L, Forte GI, Cammarata FP, Lio D, Messa C, et al. Portrait of inflammatory response to ionizing radiation treatment. Journal of inflammation (London, England). 2015;12:14.
200. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. Nature reviews Cancer. 2015;15(7):409-25.
201. Horton JA, Hudak KE, Chung EJ, White AO, Scroggins BT, Burkeen JF, et al. Mesenchymal stem cells inhibit cutaneous radiation-induced fibrosis by suppressing chronic inflammation. Stem cells (Dayton, Ohio). 2013;31(10):2231-41.
202. Furnham A, Alibhai N. Cross-cultural differences in the perception of female body shapes. Psychological medicine. 1983;13(4):829-37.
203. Bröstimplantatregistret. BRIMP THE BREAST IMPLANT REGISTRY ANNUAL REPORT 2016. https://stratum.registercentrum.se/#!page?id=1845: Kliniken för rekonstruktiv plastikkirurgi Karolinska Universitetssjukhuset; 2016.
204. Surgery ISoAP. THE INTERNATIONAL STUDY ON AESTHETIC/COSMETIC PROCEDURES PERFORMED IN 2016 https://www.isaps.org/medical-professionals/isaps-global-statistics/2016 [
205. Andersen KG, Duriaud HM, Jensen HE, Kroman N, Kehlet H. Predictive factors for the development of persistent pain after breast cancer surgery. Pain. 2015;156(12):2413-22.

206. Sarfati I, Ihrai T, Kaufman G, Nos C, Clough KB. Adipose-tissue grafting to the post-mastectomy irradiated chest wall: Preparing the ground for implant reconstruction. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2011;64(9):1161-6.
207. Delay E, Meruta AC, Guerid S. Indications and Controversies in Total Breast Reconstruction with Lipomodeling. Clin Plast Surg. 2018;45(1):111-7.

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Improved shape and consistency after lipofilling of the breast: Patients' evaluation of the outcome

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ORIGINAL ARTICLE

Improved shape and consistency after lipofilling of the breast: Patients' evaluation of the outcome

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Abstract

Autologous transplantation of fat, or lipofilling, of the breast can correct deformities after reconstruction and after conservation surgery for cancer. We have made a retrospective evaluation of morbidity and patients' experiences of the method. Forty-four patients have had lipofilling once, twice, or three times (mean transplanted volume 40 ml, range 6–200 ml). One minor complication, a local infection, was treated with antibiotics given orally, and recorded at follow up. A questionnaire was sent to all 44 patients and answered by 43. Twenty-nine of the 43 patients reported improvement of irregularities after lipofilling, 26 reported that the shape of the breast had improved, 26 that the consistency had improved, and 20 that the size had increased postoperatively. Five of the patients reported some adverse effects; one case of liponecrosis that did not need any treatment was found one year postoperatively. No problems were reported at the donor site at follow-up.

Key Words: *Lipofilling, autologous fat transplantation, fat grafting, lipomodeling, breast, breast reconstruction, breast conservation surgery*

Introduction

Transplantation of autologous fat to the breast dates back to 1895 [1] and has been increasingly used for various purposes by plastic surgeons for the last 25 years. The use of lipofilling for mammary deformities has been delayed by the possible risk of the development of microcalcifications and cysts that might mimic malignancies or delay the diagnosis of breast cancer. Palpable masses as a result of fat necrosis may also be a diagnostic problem [2]. The results after fat grafting have also been considered unpredictable because of partial reabsorption of the graft [3]. More recent papers have indicated that these problems might be of less importance than formerly believed. Good results and patients' satisfaction have been reported by several authors after correction of contour deformities in reconstructed breasts [3-9], correction of deformities after breast conservation for breast cancer [6,10], for congenital breast deformities such as Poland syndrome, for tuberous breasts [3,8,9,11], and for breast augmentation [3,4,8]. Many patients have postoperative radiotherapy for breast cancer as it considerably reduces the risk of local recurrence, but this treatment increases the risk of complications and gives a less successful aesthetic outcome after breast surgery. Lipofilling of breast reconstructions after irradiation improves the aesthetic outcome and decreases skin atrophy, oedema, and fibrosis [12]. The useful effects on the quality of tissue have been attributed to the regenerative property of autologous fat that contains adipose tissue-derived stem cells (ADSC) [5,13].

We have found no other studies that specifically addressed the patients' experience of the method. Here we describe our initial experiences from 43 patients with deformities after operations for breast cancer and radiotherapy. The patients' experiences have been evaluated using a questionnaire.

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Patients and methods

From June 2006 to March 2010 44 patients had lipofilling after mastectomy and breast reconstruction or breast conserving surgery for cancer in our department. Forty-three of the patients answered a questionnaire and their results have been evaluated. We retrospectively reviewed all the charts for the 43 patients. The patient who did not answer the questionnaire did not differ from those who answered regarding patient characteristics, previous history, or amount of injected fat. No complications were recorded in her chart.

Patients

Their mean age was 53 years (range 30–72) and the mean BMI 24.1 (range 18.5–40). Thirty-four of the patients had had mastectomies and 9 breast-conserving operations. Six of those who had had mastectomy were BRCA-1 or BRCA-2 gene mutation carriers who had had prophylactic mastectomy. Types of breast reconstruction are shown in Table I. Twenty-nine of 43 patients had had postoperative radiotherapy. The median time from the end of radiotherapy to the first lipofilling was 43 months (range 17->240). There were four smokers in the group and none of the patients had diabetes mellitus. According to the ASA classification 30 were ASA 1, 11 ASA 2, and one ASA 3.

Operative technique

The areas in the breast to be treated were marked preoperatively, with the patient standing up. Operations were done under general anaesthesia. Fat was harvested from the abdomen using a dry technique with a blunt Coleman cannula mounted on a 10 ml syringe. After harvest the fat tissue was centrifuged at 3000 rpm for 3 minutes. After the liquid fat had been disposed of, together with blood, the remaining fat was injected into the subcutaneous tissues of the breast and in some cases into the pectoral muscle using another blunt cannula with a diameter of 1.29 mm. Care was taken to inject the fat in as many thin layers as possible.

Lipofilling was combined with other surgical techniques in 27 of the 43 patients. (Table II).

Table I. Breast reconstructions before lipofilling in 34 patients.

Type of reconstruction	No. of patients	No. of patients irradiated
Expander prosthesis	21	12
Permanent prosthesis	8	4
Latissimus dorsi flap and prosthesis or expander	5	5

Six of the patients had lipofilling in both breasts, the remaining 37 patients in one. Lipofilling was required once by 22 patients, twice by 18, and three times by 3 patients. The median amount of transplanted fat per session was 40 ml (range 6–200 ml).

Questionnaire

A study-specific questionnaire was designed by the authors and sent by mail to 44 patients at two different times, in March 2009 and June 2010. Questions were asked about the consistency of the breast, the size of the breast, the shape of the breast, the sensitivity of the skin, its quality, and irregularities of the breast. Patients were asked to evaluate the result as either much improved, improved, unaltered, worse, or much worse. They were asked to report any problems with the donor area, and also about late complications, and space was left for the patient's own comments. A reminder was sent after 1–2 months. Only patients with a follow-up time of 6 months or longer were included, to make sure that a consistent result was established after initial reabsorption of fat.

Results

All patients had been seen at least once postoperatively at our outpatient clinic. Review of the patient charts showed only one postoperative complication that could be related to the lipofilling. It was a local infection/liponecrosis that had been treated with antibiotics given orally. No haematomas or fat necroses necessitating reoperations or any other treatments were recorded. There were no new lumps or other clinical problems that needed radiological evaluation during follow-up.

Thirty of the patients had visited their oncologist after the lipofilling, and all but one had normal findings on clinical examination. Sixteen of the patients had had routine ultrasonography of their breasts that showed no abnormal findings. One of the patients developed a lump one year after the operation, but at

Table II. Simultaneous operations in 27 of 43 patients (some had several operations).

Operations	No. of patients
Capsulotomy and replacement of implant	15
Correction/reconstruction of nipple	6
Excision of skin and correction of scar	6
Liposuction in the breast	3
Abdominal advancement	3
Replacement of implant	2
Correction of flap	1

ultrasonography and mammography it was diagnosed as typical liponecrosis, which required no additional examinations.

The questionnaire was answered by 43 of the 44 patients, 29 of whom reported that their irregularities had been improved by lipofilling; 26 reported improved shape, and 26 improved consistency. Increased size postoperatively was found by 20 patients; 11 had improved sensitivity of the skin and 8 improved skin quality (Figure 1). The answers from 9 patients who had lipofilling after breast conservation surgery, were studied separately. The median amount of transplanted fat per session was 46.5 ml (range 15–86) and the total injected median amount 124 (20–147) ml. These patients were more satisfied with the results of lipofilling than the breast reconstruction group (Table III).

Five patients reported adverse effects, and evaluation of these is difficult as they all had other simultaneous procedures. Three patients reported deterioration of size: two had had 17 ml and the third 55 ml. Two of them had had liposuction in other parts of the breast during the same operation to improve its shape. The same patients described deterioration of shape and irregularities. One patient found that the size was the same or a little smaller after lipofilling with 6 ml, abdominal advancement, and a change of prostheses. One patient found the shape much improved after injection of 67 ml of fat, but the sensitivity had deteriorated (Figure 1). None of the patients had any problems from the donor site at the time of the follow-up.

Four patients commented that the initial result of the lipofilling was good but that the effect gradually disappeared.

It was not possible in this limited group of patients to find out whether there was a difference in success rate in patients who have had radiotherapy and those who have not, nor was it possible to correlate results with the amount of fat that had been transplanted. It was also not possible to distinguish the effect of the lipofilling itself from the effect of simultaneous operations.

Discussion

Many authors have described good cosmetic results after lipofilling [3,4,6,8,10], which agrees with our experience. Despite the small fat volumes used in our patients most of them were satisfied. The most common good effect reported by the patients themselves was improved shape, including improvement in irregularities of the breast and consistency. The improved consistency might, at least in part, be attributed to the fact that 29 of 43 patients have undergone radiotherapy. It has previously been shown that radiotherapy-induced tissue damage can improve after lipofilling [5,13]. The regenerative property of the injected fat is thought to be mediated by stem cells derived from adipose tissue that promote neovascularisation of ischaemic tissues [13,14]. Improved shape and reduced irregularities of the breast were also reported by most of our patients, which were the primary indications for lipofilling in most of them. Injection of relatively small fat transplants can correct contour deformities that are important for cosmesis, particularly in the upper medial part of the breast. (Figures 2–4).

In 2007 Missana et al. reported results after lipofilling in a group of 69 patients including 9 who had had breast conservation after breast cancer. Results

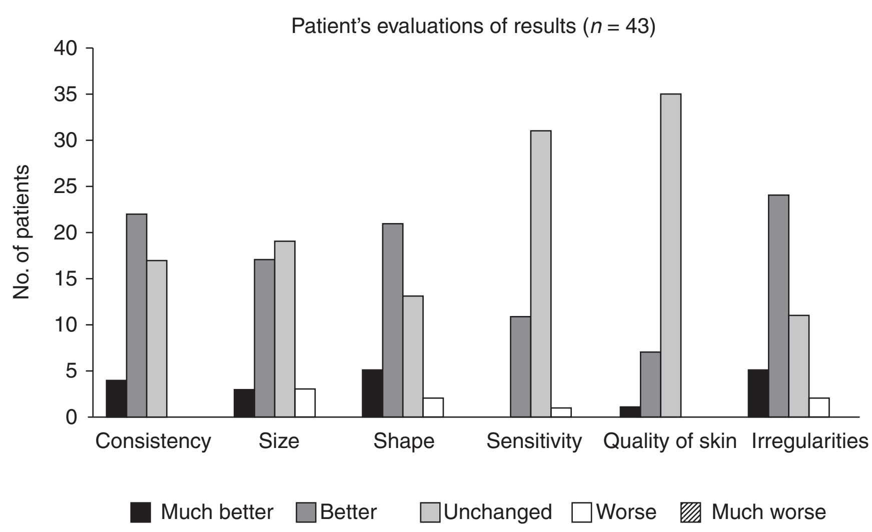

Figure 1. Patients' evaluation by questionnaire of the outcome after lipofilling.

Table III. Patients' evaluation of results.

	After breast conservation (n = 9)			After breast reconstruction (n = 34)		
	Better	Unchanged	Worse	Better	Unchanged	Worse
Consistency	6	3		20	14	
Size*	7	2		13	17	3
Shape**	7	2		19	11	2
Sensitivity	2	6	1	9	25	
Quality of skin	4	5		4	30	
Irregularities*	7	2		22	9	2

*One additional patient answered "unchanged-worse". **One additional patient answered "unchanged-worse" and one did not answer.

were considered promising and, in their opinion, lipofilling is a good compromise between no operation and major reconstruction [6]. Similar experiences have been reported by Delay et al. who did lipofilling in 42 patients after breast conservation after cancer [10]. In the present study defects after breast conservation were treated in 9 patients. This group of patients expressed greater satisfaction with the results than did the patients who had lipofilling after breast reconstruction. This finding might reflect that the reconstructed patients' answers reflected satisfaction with the breast reconstruction as well as the lipofilling, making results in the two different patient groups difficult to compare.

Postoperative reabsorption of injected fat is a well-known problem after lipofilling [1,15]. Some authors recommend overcorrection [16] while others prefer several sessions of lipofilling [3]. Delay et al. have a vast experience of fat transplantation to the breast and consider that 30%-40% of the injected fat is reabsorbed and so overcorrect if possible [9]. Four of our patients specifically commented on the deterioration of the initial result. It is probable that greater volumes or several sessions, or both, would have produced more evident results.

We found only one postoperative complication during follow-up that could be related to the lipofilling, but one patient later developed a small palpable lump in the same area where she had had lipofilling. After mammography and ultrasonography it was considered typical liponecrosis that did not require treatment or further examination. These

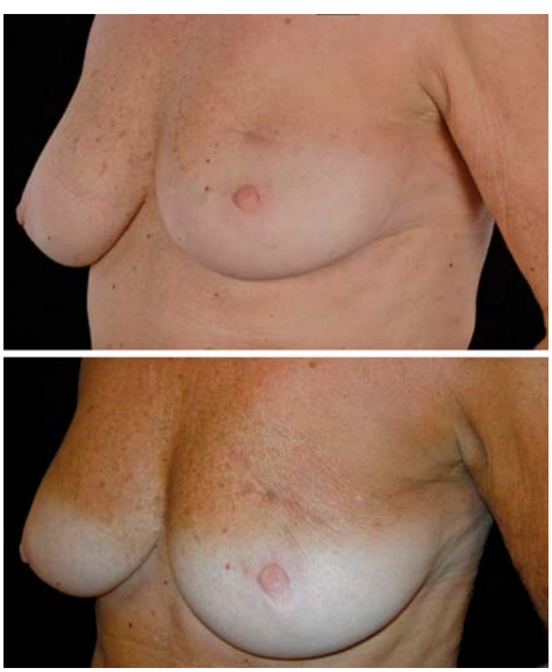

Figure 2. Before (top) and after (below) one session of lipofilling with fat 34 ml 5 years after breast conservation for cancer and radiotherapy.

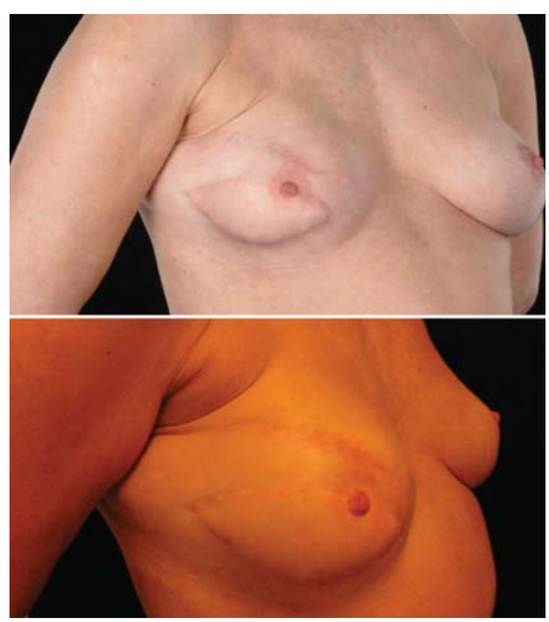

Figure 3. Before (top) and after (below) 2 sessions of lipofilling (total 102 ml) to the right breast. The patient has had her breast conserved and radiotherapy of the right breast, followed by bilateral mastectomy. The right breast was reconstructed using a latissimus dorsi flap and a prosthesis 16 months before the first lipofilling.

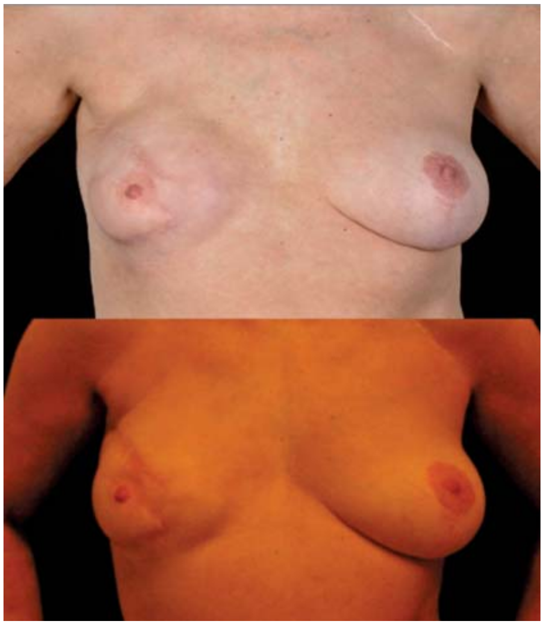

Figure 4. Same patient as Figure 3. Before (top) and after (below) 2 sessions of lipofilling (total 102 ml) to the right breast.

findings are similar to others. Spear et al. reported four complications in 47 treated breasts with a mean follow-up of 49 weeks, one cellulitis, and 3 small liponecrotic cysts [4], Missana et al. reported five cases of liponecrotic cysts but no early complications after 74 procedures with 11.7 months follow-up [6], and Rietjens et al. 7 minor complications in 194 fat grafting procedures [17]. Rietjens et al. evaluated mammograms in 77 patients having had breast conserving cancer surgery and lipofilling. Four of these patients had postoperative changes after lipofilling, all classified as benign that did not need further investigations. The liponecroses and other mammographic findings developed gradually and it is important that the radiologist should be informed about lipofilling. Risk of delaying breast cancer detection was the reason for the 1987 statement of the American Society of Plastic and Reconstructive Surgeons deploring the use of autologous fat transfer to the breast [2]. Since then several reports have described changes seen at mammography, ultrasonography, and MRI after lipofilling [3,17–20]. Their conclusion is that radiological changes seen after lipofilling can be differed from malignant findings by an experienced radiologist.

We consider lipofilling to be a good method for improving results after breast reconstructions and after breast conservation for breast cancer but long-term effects of changes caused by injection of fat into the breast remain to be studied.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg 1989;83:368–81.

[2] Report on autologous fat transplantation. ASPRS Ad-Hoc Committee on New Procedures, September 30, 1987. Plast Surg Nurs 1987;7:140–1.

[3] Illouz YG, Sterodimas A. Autologous fat transplantation to the breast: a personal technique with 25 years of experience. Aesthetic Plast Surg 2009;33:706–15.

[4] Spear SL, Wilson HB, Lockwood MD. Fat injection to correct contour deformities in the reconstructed breast. Plast Reconstr Surg 2005;116:1300–5.

[5] Panettiere P, Marchetti L, Accorsi D. The serial free fat transfer in irradiated prosthetic breast reconstructions. Aesthetic Plast Surg 2009;33:695–700.

[6] Missana MC, Laurent I, Barreau L, Balleyguier C. Autologous fat transfer in reconstructive breast surgery: indications, technique and results. Eur J Surg Oncol 2007;33:685–90.

[7] Coleman SR. Structural fat grafting: more than a permanent filler. Plast Reconstr Surg 2006;118(suppl 3):108S–20S.

[8] Coleman SR, Saboeiro AP. Fat grafting to the breast revisited: safety and efficacy. Plast Reconstr Surg 2007; 119:775–87.

[9] Delay E, Garson S, Tousson G, Sinna R. Fat injection to the breast: technique, results, and indications based on 880 procedures over 10 years. Aesthet Surg J 2009;29:360–76.

[10] Delay E, Gosset J, Toussoun G, Delaporte T, Delbaere M. [Efficacy of lipomodelling for the management of sequelae of breast cancer conservative treatment]. Ann Chir Plast Esthet 2008;53:153–68.

[11] Pinsolle V, Chichery A, Grolleau JL, Chavoin JP. Autologous fat injection in Poland's syndrome. J Plast Reconstr Aesthet Surg 2008;61:784–91.

[12] Serra-Renom JM, Munoz-Olmo JL, Serra-Mestre JM. Fat grafting in postmastectomy breast reconstruction with expanders and prostheses in patients who have received radiotherapy: formation of new subcutaneous tissue. Plast Reconstr Surg 2010;125:12–18.

[13] Rigotti G, Marchi A, Galie M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 2007;119:1409–24.

[14] Borges J, Torio-Padron N, Momeni A, Mueller MC, Tegtmeier FT, Stark BG. Adipose precursor cells (preadipocytes) induce formation of new vessels in fibrin glue on the newly developed cylinder chorioallantoic membrane model (CAM). Minim Invasive Ther Allied Technol 2006;15:246–52.

[15] Locke MB, de Chalain TM. Current practice in autologous fat transplantation: suggested clinical guidelines based on a review of recent literature. Ann Plast Surg 2008;60:98–102.

[16] Chajchir A, Benzaquen I, Wexler E, Arellano A. Fat injection. Aesthetic Plast Surg 1990;14:127–36.
[17] Rietjens M, De Lorenzi F, Rossetto F, et al. Safety of fat grafting in secondary breast reconstruction after cancer. J Plast Reconstr Aesthet Surg 2011;64:477–83.
[18] Hyakusoku H, Ogawa R, Ono S, Ishii N, Hirakawa K. Complications after autologous fat injection to the breast. Plast Reconstr Surg 2009;123:360–72.
[19] Pierrefeu-Lagrange AC, Delay E, Guerin N, Chekaroua K, Delaporte T. [Radiological evaluation of breasts reconstructed with lipomodeling]. Ann Chir Plast Esthet 2006; 51:18–28.
[20] Pulagam SR, Poulton T, Mamounas EP. Long-term clinical and radiologic results with autologous fat transplantation for breast augmentation: case reports and review of the literature. Breast J 2006;12:63–5.

Improved Patient-Reported Outcomes after Autologous Fat Transplantation and corrective surgery after Breast Surgery

Authors

Anna Lindegren, Inkeri Schultz, Marie Wickman Chantereau

Background

Autologous fat transplantation (AFT) is being increasingly used to improve the results after breast-conserving surgery and breast reconstruction. However, studies on patient-reported outcome (PROs) and health-related quality of life (HRQoL) after AFT are scarce. The aim of this prospective longitudinal case-series study was to assess PRO in women who had undergone AFT after surgery for breast cancer or risk-reducing mastectomy.

Methods

Fifty women, who had undergone breast-conserving surgery or breast reconstruction, needing corrective surgery, were consecutively included between 2008 and 2013. A 20-item study-specific questionnaire (SSQ) and the Short Form Health Survey (SF-36) were used pre-operatively and six months, one year and two years post-operatively, to evaluate PRO and HRQoL.

Results

The patients underwent three (1-4) AFT procedures, with the injection of 164 (median) (range 40-516) ml fat. Thirty-eight and 34 patients completed the study-specific questionnaire and the SF-36 respectively, both pre-operatively and after two years. Sixteen of the 20 items in the SSQ were improved after two years, including breast size ($p<0.0001$), shape ($p<0.0001$), appearance ($p<0.0001$), softness of the breast ($p=0.001$), pain in the region ($p=0.005$), scarring from previous breast surgery ($p<0.001$) and willingness to participate in public physical activities ($p<0.001$). HRQoL did not largely differ before and after AFT, or between the study group and a reference population.

Conclusions

AFT alone or in combination with other corrective surgical procedures, improved PRO after breast-conserving surgery and breast reconstruction in both irradiated and non-irradiated women.

Introduction

Breast reconstruction in connection with, or after, breast cancer surgery is well known to improve quality of life[1-3]. The surgical methods employed range from an oncoplastic approach in breast cancer surgery to extensive free-flap reconstructions. All have in common the possible need for surgical correction due to complications or less favourable aesthetic outcome. Patients who have received radiotherapy of the breast or the chest wall have a higher frequency of complications and reoperation[4]. Common indications for reoperation due to unfavourable aesthetic results are indurations, scar contractures, tissue deficit and breast asymmetry. Autologous fat transplantation (AFT) has become a well-established technique for improving these conditions[5]. Clinical results and surgeon's opinions about the outcome after AFT are generally reported as good[6-9]. The results of some studies indicate that surgeon-reported outcome and patient-reported outcome may diverge after breast reconstruction[10-13]. The results of these evaluations can be valuable for patients facing quality-of-life-improving surgery in their decision-making process. There is also an increasing interest in patient reported outcome measures (PROMs) for the evaluation of the efficacy of the health care[14-16].

In most studies on patients' opinions of the outcome of reconstructive surgery only a single question has been asked [8, 9, 11, 17]. We have previously reported patients' satisfaction after AFT in a questionnaire-based, descriptive cross-sectional study[18]. The aim of the present study was to assess patient reported outcome after AFT, prospectively and longitudinally, as part of reconstructive breast surgery. We also wanted to assess the patients' health-related quality of life (HRQoL) before and after surgery, and learn if it is consistent with HRQoL of a Swedish reference population.

Materials and Methods

Patients and Design

The study design is a prospective case series including patients treated with AFT as part of corrective surgery at the Department of Reconstructive Plastic Surgery at Karolinska University Hospital in Stockholm, Sweden. The patients were consecutively enrolled from October 2008 until June 2013. All procedures were performed under the national health service. Those included were women who had undergone breast-conserving surgery with less favourable aesthetic results, and women who had undergone mastectomy and subsequent breast reconstruction, needing corrective surgery. The inclusion criteria were: time after previous breast surgery or radiotherapy longer than one year, and mammography and ultrasound examinations performed a maximum of three months prior to AFT, to exclude any local recurrences. Exclusion criteria were: known breast cancer recurrence, contraindications for anaesthesia or surgery due to comorbidities, other serious systemic diseases that may affect the outcome of the procedure, and inability to understand verbal and written information about the study. The patients' characteristics are given in Table 1. The study was approved by the Regional Ethics Review Board in Stockholm (2008/484-31/2).

Autologous Fat Transplantation

Fat aspiration was performed with a dry technique using a Coleman cannula and a 10 ml syringe at the donor site[19]. Donor sites were the abdomen, knees, thighs, hips, flank area, in one case arms, and in another the case sub-axillary region. The fat was centrifuged at 3000 rpm for three minutes. Liquid fat and blood were poured off, and the remaining lipocytes were injected into the subcutaneous tissues at the recipient site in multiple layers and directions using a blunt cannula, 1.29 mm in diameter. Patients with implants received one dose of prophylactic antibiotics (cloxacillin provided they were not allergic, otherwise clindamycin). The patients underwent a median of three (1-4) AFT procedures unilaterally or bilaterally until satisfactory results were obtained. Other corrective procedures were performed concurrently in eleven patients: seven scheduled nipple reconstructions, one mastopexy, two scar corrections and one capsolutomy with implant change, abdominal advancement and liposuction of the axilla. The median time from the first AFT to last AFT was 12.3 months (range 4.2-27.5). All operations were performed under general anaesthesia and carried out by one of the authors.

Questionnaires and Data Collection

Demographic data were collected from electronic patient medical files that cover the whole catchment area. Hence, additional operations performed outside the unit and any complications treated at other hospitals would come to our knowledge. A study-specific questionnaire (SSQ) modified for this specific study population, was used. The original questionnaire that SSQ was based on was developed by a psychologist [20], specialised in oncological patients, in collaboration with experienced plastic surgeons. It has been consequently modified for different studies focusing on other breast reconstructive methods and their outcome[21, 22], This SSQ contains 16 items in which answers are given on a seven-point Likert scale (Q1-Q4 & Q9-Q20), and four items (Q5-Q8) where the responses are 'Not at all', 'Little', 'Pretty much' or 'Much'. The BREAST-Q questionnaire was not available at the start of this study.

The Short Form Health Survey[23] (SF-36) measures the patients' long-term (four weeks) HRQoL. It covers eight domains: physical functioning, physical role functioning, bodily pain, general health, vitality, social functioning, emotional role functioning and mental health in 36 items. The mean scores for each domain were transformed into a value on a 0 to 100 scale, where a higher number represents higher functioning. The eight domains of the SF-36 were used to compare the patients' HRQoL with that of a Swedish reference population, change over time within the group. The outcome of the reference population is provided in the Swedish translation of SF-36 and based on answers from 3260 randomly picked women aged 30-74 in seven Swedish regions during 1991-92.

The questionnaires were answered at baseline (before AFT) and six months, one year and two years after the final AFT, either by mail or at the outpatient clinic. Median follow-up (time from final AFT procedure to last completed questionnaire) was 24.2 months (range 11.8-32.3). The completed questionnaires were handled by the first author and not by the surgeon who performed the operations.

Statistical Methods

The items on an interval scale in the SSQ (Q1-Q4 & Q9-Q20) were visualized using descriptive statistics and mean line graphs were plotted using the results from all SSQs from all patients (n=48), to give an overview of the change over time for each question. The items were also analysed using a statistical model, Mixed-effect model repeated measures (MMRM), to assess the longitudinal data. This model incorporates the dependence of the measurements within a patient and allows missing data (i.e. SSQ not completed at six months and/or one year) to analyse only the answers from the patients who completed both the baseline and the two-year SSQ (n=38). The model generates an estimate of the mean change. The outcome variable was the change relative to the baseline, and the baseline value was included as a covariate in the model. Sensitivity analysis was performed for irradiated and non-irradiated patients. This was carried out to examine if AFT especially benefits the irradiated patients. It is not inconceivable since AFT is known to have a positive impact on irradiation-damaged tissue[24]. Moreover, sensitivity analysis was performed for patients

(92%) that had not undergone major additional surgery. The limit for the confidence interval was 95 % for the difference between each time point and the baseline.

In the ordinal items (Q5-Q8), a quasi-symmetry model[25] was used to estimate the probability that a patient would score higher after two years than before surgery. Since the responses were given on an ordinal scale, they cannot be treated as continuous variables in the analyses. The model acknowledges the ordinal scale and tests for an ordinal trend.

The level of significance was set to 5 % ($p \leq 0.05$) in all tests. The statistical analyses were performed using SAS® (Version 9.4, SAS Institute Inc., Cary, NC, USA). No imputations were performed and no last observation carried forward was used.

No statistical calculations were performed since the number of patients only allow for detection of 10-20 point differences both over time within the group and between the patients and the reference population. Mean scores for the general Swedish population are given by sex and age categories in the Swedish translation of SF-36. The scores for women in the nine age categories between 30 and 74 years were used to calculate a mean score for all the women aged 30-74 (n=3260). The means were weighted with respect of number of patients in each age category. In that way the reference population and patients are matched on sex and age. Confidence interval for the computed mean for the reference population was not calculated.

Results

Patients

Fifty patients were recruited to the study. One patient died and one dropped out before completing any questionnaires. Among the remaining 48 patients, eight dropped out during the study period, but their completed questionnaires were analysed. One dropped out because of breast cancer recurrence, one underwent bilateral breast augmentation at a private clinic, one bilateral risk-reducing mastectomy, and five patients dropped out for other reasons (Figure 1). Two other patients had breast cancer recurrence, but they did not drop out of the study.Adverse events occurred in six patients: one patient had a wound rupture, one a haematoma at the donor site, and four patients needed further investigations; three because of palpable lumps and one because of discomfort and swelling of the breast. Thirty-eight patients completed the SSQ and 34 patients completed the SF-36 both at baseline and after two years.

The total median volume of injected fat was 164 ml (40-516).

Study-Specific Questionnaire

Q1-Q4 and Q9-Q20

From the mean line graphs it can be seen that the best results were obtained after six months, followed by a decrease after one year, and then an increase after two years (Figure 2). Fourteen of these 16 items (Q1, Q4 & Q9-Q20) were improved after two years. The mean score and mean change between baseline and two years are given for each item in Table 2 and Figure 2.

In the MMRM analysis, including only patients who completed both baseline and the two-year questionnaire, thirteen of the 16 items were significantly improved after two years, for the entire group and in the sensitivity analyses for the irradiated patients and the patients who had not undergone additional surgery. (Q4 & Q9-Q20). Q13 (softness of the breast) was not significantly improved after one year (Table 3). The non-significant items (Q1-Q3) concern the sensitivity of the breasts. The non-irradiated subgroup was too small to make any inferences from the data.

Q5-Q8

The ordinal trend, i.e., the probability of more positive scores after two years, was 22.47 ($p=0.002$) in Q6 (sexually unattractive because of breasts), 7.9 ($p=0.003$) in Q7 (seeing oneself naked), and 10.88 ($p=0.017$) in Q8 (being naked in front of partner) (Figure 3). The 95 % CI was wide in all three items. The ordinal trend for Q5 (importance of breasts for sexuality) was not significant.

SF-36

The mean scores for the eight domains for the reference population were compared with the mean score for the study group at both baseline and after two years. No point differences were large enough to be able to detect any significant differences in HRQoL between the study group and the reference population. Neither were there any large differences between the mean score at baseline and at the two-year follow-up within the study group. The descriptive statistics are shown in Figure 4.

Discussion

Patient-reported outcome measures are important tools in evidence-based medicine[26, 27]. The results are especially important when evaluating surgery intended to improve quality of life, rather than treating severe health conditions. A debate on oncological safety has been going on over the last three decades. It has been proposed that stem cells injected in a previous cancer site could induce recurrence[28]. Consequently, women offered AFT as part of breast reconstruction or after breast-conserving surgery should be given evidence-based information about the outcome of the procedure before accepting further surgery. Information on the outcome of different procedures is also important for health-care providers and funding bodies. Despite the common use of AFT, few reports have been published on the change of patient reported outcome over time and the quality of the reports is rather low[26]. When this study started, the validated BREAST-Q form was not yet available, and could therefore not be used as recently recommended in the VOGUE study[27]. Instead a study-specific questionnaire was used to evaluate patient reported outcome. The patients answered the questionnaire before AFT, and at three time points during the two-year follow-up after completed AFT sessions. A research nurse handled the questionnaires. The aim was to assess possible changes in the patients' opinion about the outcome over time. Resorption of the transplanted fat may lead to deterioration of the aesthetic result over time. On the other hand, as time passes, the patients may accept the cosmetic result to a larger extent and softening of the breast tissue[29] could lead to patients expressing a more positive opinion over time.

In the present study, a more positive outcome was reported in 80 % of the items two years after AFT, than pre-operatively. The patients were more satisfied with the appearance of their breast with and without clothes, found the breast symmetry better, were more comfortable about being naked with their partner, and felt more sexually attractive. They were also more willing to participate in physical activities, and found it easier to find a good-fitting bra. The relatively long period between radiotherapy and AFT (median >3.5 years, extending to over 22 years) indicates that AFT could the main reason for improved scoring post-operatively. If that period had been shorter, the initial negative impact of radiotherapy would decrease during the study period, making time the main reason why the patients have higher scores after two years. Eleven of the patients had other ipsilateral surgical procedures performed. Seven of these were elective nipple operations leaving four patients who underwent surgery that could have positive or negative impact on the outcome. Only three of them were included in the MMRM analysis. Since these three patients had to undergo additional surgery one can speculate if they have scored more negatively. On the other hand the additional surgery may have generated even better outcome. The patients had increased in BMI from 24.7 to 25.3 kg/m^2 in median at the last follow-up. It is less likely that the small increase have an impact on the outcome.

Various aspects of AFT have been studied, but the number of studies using PROMs is modest. Most publications report only a one-item evaluation, asking the patient if she is satisfied with the outcome, often using a 3- to 5-point Likert scale[8, 9, 17, 30]. In one study, 200 patients were asked personally about their degree of satisfaction with the surgical outcome at their post-operative follow-up. All of them claimed that they were satisfied or very satisfied, which may imply a significant systematic bias as the evaluation was not anonymous[31]. In the present study, care was taken to prevent the surgeons involved from handling the post-operative questionnaires, so as not to influence the outcome measures.

In the only multi-centre cohort study on this subject, Bennett et al.[32], reported that AFT patients and 'control patients' scored similarly at baseline regarding physical well-being, satisfaction with the breast, sexual well-being and psychosocial well-being. More over the control patients scored a little higher than the AFT patients (cases) at the one-year follow-up. No difference between the two groups was seen after two years. However, almost 20 % of the control patients had undergone AFT before baseline, which may lead to problems in the interpretation of the results. Moreover, no comparison was made of pre- and post-operative results. Nevertheless, the authors concluded that AFT provided measurable improvements in satisfaction, which is in line with our results.

In the present study, the nine items that can be regarded as indicators of aesthetic outcome and symmetry were all improved. The patients' opinion of the aesthetic outcome could therefore be considered improved, which was one of the main reasons for performing AFT (Figure 5 and 6). It is difficult to assess the impact of breast surgery on sexuality, but this is important for the patient's future well-being. Many breast cancer survivors suffer from impaired sexuality due to a change in body image[33]. The results of this study indicate an improvement over time after AFT regarding feeling sexual attractive and comfortable when being naked. Whether this is due to AFT or the passage of time after cancer surgery and reconstruction is unclear. Our findings are in contrast to those in the study by Bennett et al., which showed a decrease in sexual well-being over time[32]. However, we believe that the use of AFT as a complement to other breast surgical techniques may play a role in regaining a positive body image after surgery.

Improvement in softness of the breast is most evident at six months and two years in the mean line plots (Q13). A non-significant change in softness was seen after one year, in contrast to two years, indicating a slightly harder breast after one year. This could be explained by a reduction in the initial filling effect over the first year due to resorption of the transplanted fat. The improvement two years post-operatively could be a biological effect in radiation-damaged tissue, rather than primarily the effect of the filler. AFT has been suggested as a treatment for radiation-induced fibrosis, as clinical findings and experimental studies indicate reduced fibrosis when AFT is carried out after radiation therapy[24, 34-37].

The patients in the present study reported a low level of pain before AFT. Despite this, reduced pain in the breast region was reported at the two-year assessment. This finding, as well as increased breast softness, is in line with the results of a case–control study carried out by Panettiere et al.[38], and implies that AFT not only improves the aesthetic outcome of a breast reconstruction, but may also have a positive impact on morbidity after treatment for breast cancer.

No large difference was found in HRQoL between the study group and the reference population, either at baseline or after two years. There is, therefore, no reason to believe that the patients in the study group differ from an age matched Swedish women in general when they decide to undergo AFT. Furthermore, it appears that AFT does not lead to a large decrease in HRQoL post-operatively.

The strengths of this study include the prospective design, with baseline data collected pre-operatively. However, the study has some limitations. Firstly, the study group is relatively small. Furthermore, additional ipsilateral surgical procedures were performed at the same time in a minority of the patients, and this could have influenced their opinions concerning the result. However, the study group represents typical patients who undergo complementary AFT. Another limitation is that the heterogeneity of the patient group may have effect on the results. A sensitivity analysis was carried out and no difference in significance was seen comparing results from the entire patient group with the sub-group (92% of the patients) that

had not undergone major additional ipsilateral surgery. No measurements of remaining fat volume were made post-operatively to compare with PRO.
A validated PROM would probably have made the evidence stronger but as mentioned before, no such was available at the start of the study. Lastly, several hypotheses tests were performed hence type I errors may exist.

In conclusion, the patient reported outcome improved up to two years after AFT, when the method was used as a complement in breast reconstruction or after breast-conserving surgery. Improved outcomes included better breast symmetry, size, shape and appearance. Autologous fat transplantation also increased the softness of the breast and reduced pain in the breast area. AFT did not affect HRQoL, and the study population did not differ from the reference population in this respect. The results of this study are of clinical importance for women considering AFT, to help them to make an informed decision. Nevertheless, further studies using PROMs after AFT and breast reconstructive surgery would be valuable to confirm our findings.

Declaration of Interest Statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. Patient characteristics

Age at baseline (y)	52.7* (31.1-71.3)
BMI at baseline (kg/m2)	24.7* (17-32.4)
BMI at follow-up (kg/m2)	25.3* (18.8-34.3)
Previous breast surgery (n)	48
Breast-conserving surgery	25
Mastectomy + Immediate reconstruction with prosthesis	10
Mastectomy + Delayed reconstruction with prosthesis	2
Mastectomy + DIEP	1
Mastectomy + LD	7
Prophylactic mastectomy + Immediate reconstruction with prosthesis	2
Prophylactic mastectomy + Immediate reconstruction with prosthesis†	1
Radiotherapy (n)	38
Time from radiotherapy to AFT (y)	3.6* (1.1-22.1)

n=48, DIEP=Deep Inferior Epigastric Perforator Flap, LD=Latissimus Dorsi Flap, *Median, †Previous cancer surgery

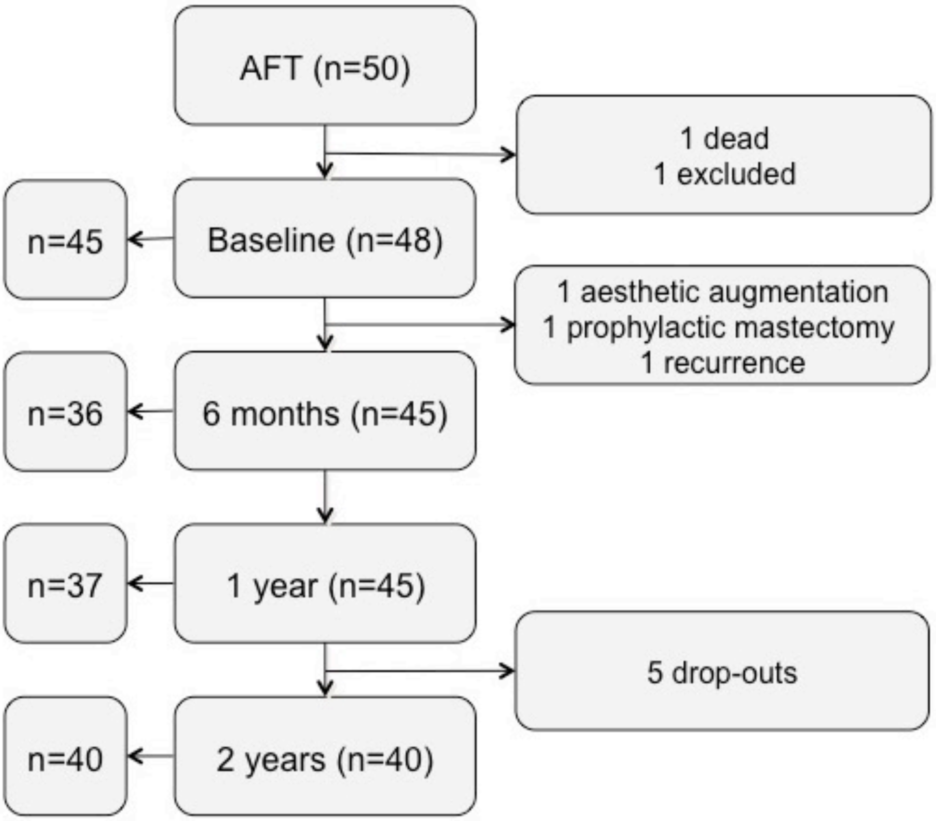


Figure 1. Flow diagram of patients' participation in the study. Boxes on the left show the number of patients completing the study-specific questionnaire (SSQ) at each time point. Boxes in the middle show the number of patients participating in the study. Boxes on the right show the number of patients excluded and drop-outs.

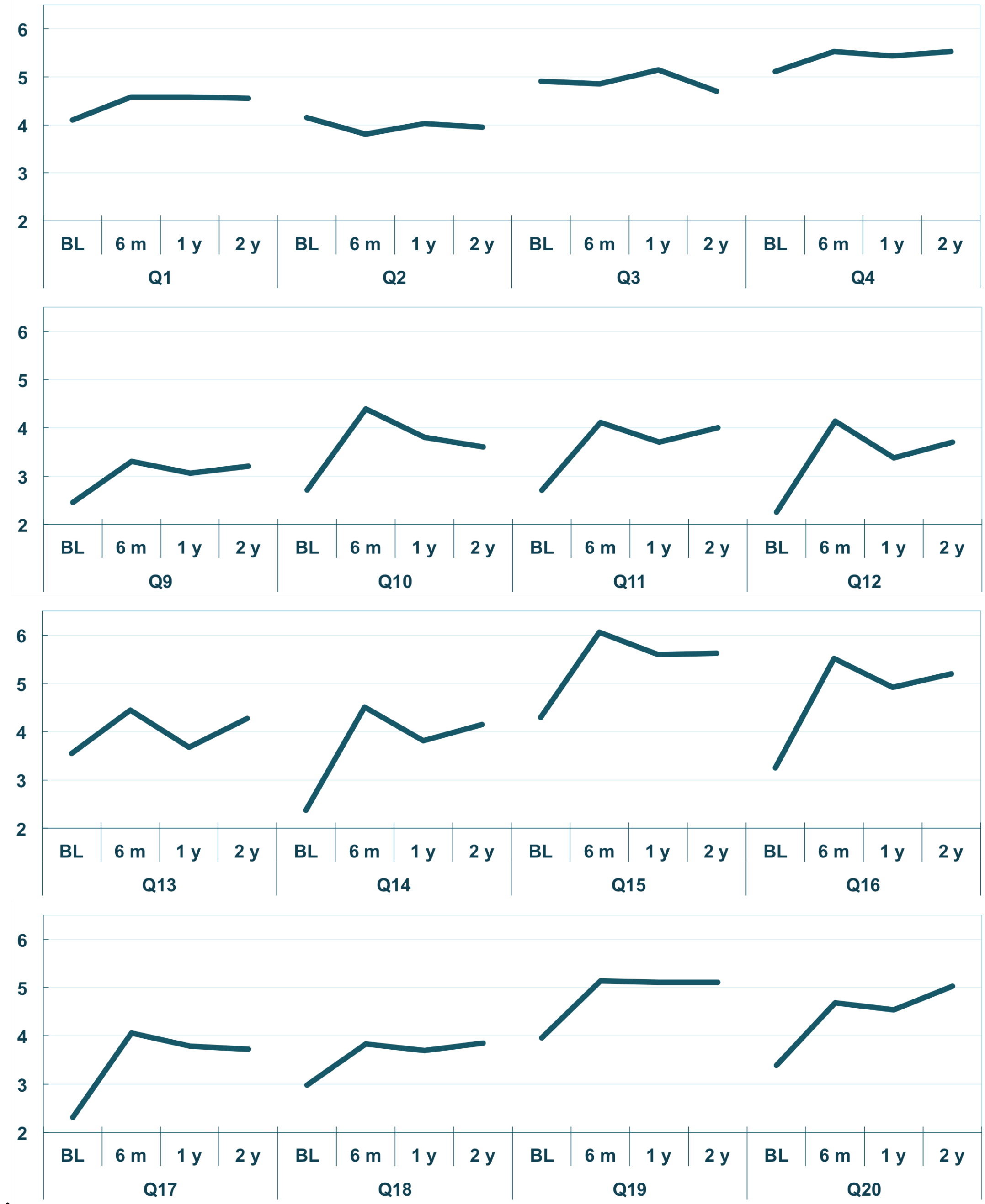


Figure 2. Mean line plots for Q1-Q4 and Q9-Q20. The mean line plots for each item at each time point are shown on the right for all patients who completed the SSQ

Table 2. Results for Q1-Q4 and Q9-Q20 on sensitivity, pain, appearance, softness, trouble finding a good-fitting bra and willingness to participate in physical activities.

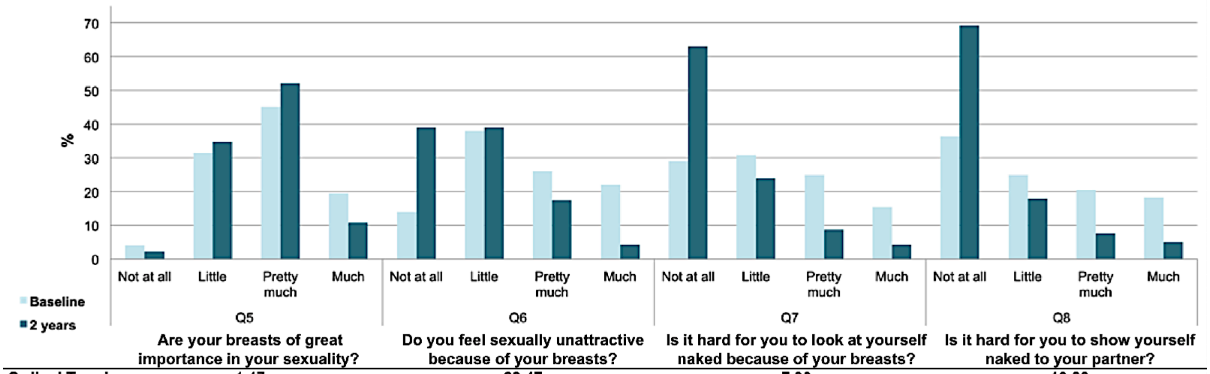
		1	... 7	BL	2 years	Min	Max	SD
Q1	How is the sensation in the nipple area?	Normal	Reduced	4.10	4.55	-3.0	5.0	1.5
Q2	How is the sensation in the breast skin?	Normal	Reduced	4.16	3.95	-4.0	3.0	1.6
Q3	How is your ability to feel sexual sensation in the breasts?	Normal	Reduced	4.91	4.70	-5.0	5.0	1.8
Q4	Do you have pain in the breasts/breast region?	Every day	Never	5.11	5.53	-3.0	4.0	1.7
Q9	What do you think about the size of your breasts?	Too small	Too big	2.45	3.21	-2.0	4.0	1.4
Q10	Are the breasts of equal size?	Very dissimilar	Very similar	2.71	3.60	-3.0	5.0	2.0
Q11	What do you think of the shape of your breasts?	Very ugly	Very nice	2.70	4.00	-3.0	5.0	2.0
Q12	Are the breasts of equal shape?	Very dissimilar	Very similar	2.25	3.70	-2.0	5.0	1.7
Q13	What do you think about the softness of your breasts?	Very hard	Very soft	3.55	4.28	-2.0	4.0	1.5
Q14	Are you satisfied with the appearance of your breasts?	Not at all	Very satisfied	2.37	4.15	-1.0	6.0	1.6
Q15	What do you think about the appearance of the breast when you wear clothes?	Very ugly	Very nice	4.30	5.63	-3.0	5.0	1.5
Q16	What do you think of the breast's appearance when you are wearing swimwear / a bra?	Very ugly	Very nice	3.25	5.20	-1.0	6.0	1.6
Q17	What do you think of the breast's appearance when you are naked?	Very ugly	Very nice	2.31	3.72	-2.0	5.0	1.7
Q18	What do you think of the scars from previous cancer surgery?	Very ugly	Very nice	2.98	3.85	-3.0	5.0	1.8
Q19	Do you have trouble finding a bra that fits your breasts?	Very hard	Not hard at all	3.96	5.11	-4.0	6.0	2.2
Q20	Do your breasts affect your willingness to swim in public environments (swimming pool, beach, etc.) or participate in gymnastics and similar activities?	Negative	Positive	3.39	5.03	-2.0	5.0	1.8

The questions were answered on a 7-point Likert scale. Mean score at baseline (BL) and two-year follow-up are shown. Min=minimum change in score between baseline and 2 years. Max=maximum change in score between baseline and 2 years. Negative numbers indicate lower score at the two-year follow-up compared to baseline. SD=Standard deviation in the change between baseline and 2 years.

Table 3. Q1-Q4 and Q9-Q20.

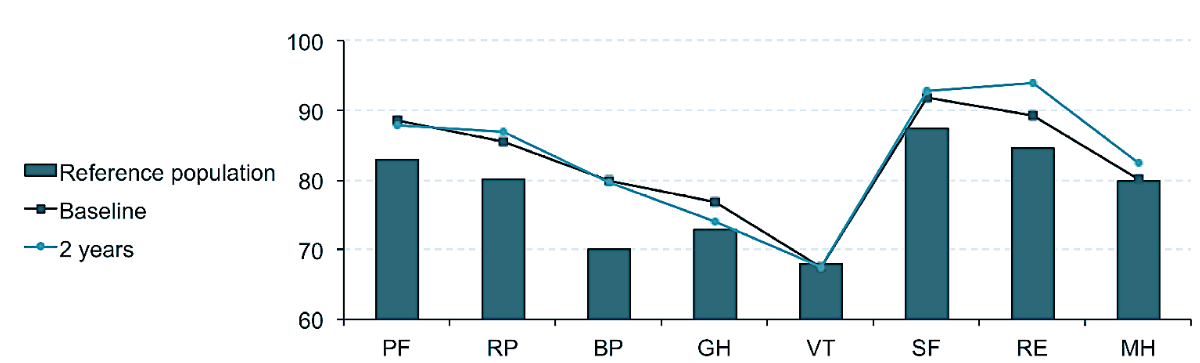
	1 year follow-up				2 years follow-up				Radiotherpy 2 years follow-up				No additional surgery 2 years follow-up			
	ECh	SE	p	CI	ECh	SE	p	CI	ECh	SE	p	CI	ECh	SE	p	CI
Q1	0.3	0.25	0.190	-0.17 - 0,83	0.3	0.23	0.160	-0.13 - 0.80	0.4	0.27	0.190	-0.18 - 0.89	0.4	0.24	0.152	-0.13 - 0.84
Q2	-0.2	0.24	0.405	-0.68 - 0,28	-0.2	0.22	0.295	-0.68 - 0.21	-0.4	0.23	0.077	-0.86 - 0.04	-0.3	0.23	0.269	-0.71 - 0.20
Q3	0.2	0.29	0.479	-0.37 - 0,78	-0.2	0.26	0.451	-0.71 - 0.32	0.0	0.29	0.999	-0.57 - 0.57	-0.1	0.27	0.621	-0.66 - 0.40
Q4	**0.5**	**0.22**	0.021	**0.08 - 0,97**	**0.6**	**0.22**	**0.005**	**0.19 - 1.05**	**0.6**	**0.24**	**0.014**	**0.13 - 1.09**	**0.6**	**0.23**	**0.015**	**0.12 - 1.02**
Q9	**0.5**	**0.17**	0.003	**0.18 - 0,85**	**0.7**	**0.16**	**<0.001**	**0.34 - 0.98**	**0.7**	**0.18**	**0.001**	**0.29 - 1.01**	**0.8**	**0.17**	**<0.001**	**0.41 - 1.10**
Q10	**1.0**	**0.26**	**<0.001**	**0.53 - 1,56**	**0.8**	**0.25**	**0.002**	**0.29 - 1.27**	**0.8**	**0.26**	**0.005**	**0.23 - 1.28**	**0.9**	**0.26**	**0.001**	**0.37 - 1.40**
Q11	**0.9**	**0.24**	**<0.001**	**0.43 - 1,40**	**1.1**	**0.23**	**<0.001**	**0.66 - 1.59**	**1.2**	**0.26**	**<0.001**	**0.73 - 1.76**	**1.0**	**0.25**	**<0.001**	**0.52 - 1.51**
Q12	**1.0**	**0.26**	**<0.001**	**0.48 - 1,52**	**1.3**	**0.25**	**<0.001**	**0.77 - 1.76**	**1.4**	**0.27**	**<0.001**	**0.82 - 1.92**	**1.3**	**0.27**	**<0.001**	**0.80 - 1.86**
Q13	0.4	0.24	0.114	-0.09 - 0,86	**0.8**	**0.23**	**0.001**	**0.32 - 1.22**	**0.9**	**0.24**	**<0.001**	**0.45 - 1.40**	**0.8**	**0.24**	**0.001**	**0.31 - 1.26**
Q14	**1.3**	**0.22**	**<0.001**	**0.87 - 1,77**	**1.7**	**0.22**	**<0.001**	**1.24 - 2.10**	**1.6**	**0.23**	**<0.001**	**1.14 - 2.07**	**1.6**	**0.20**	**<0.001**	**1.15 - 1.97**
Q15	**1.5**	**0.21**	**<0.001**	**1.10 - 1,93**	**1.3**	**0.20**	**<0.001**	**0.95 - 1.75**	**1.5**	**0.19**	**<0.001**	**1.14 - 1.91**	**1.2**	**0.20**	**<0.001**	**0.82 - 1.62**
Q16	**1.9**	**0.23**	**<0.001**	**1.40 - 2,32**	**2.0**	**0.22**	**<0.001**	**1.57 - 2.45**	**2.0**	**0.22**	**<0.001**	**1.55 - 2.45**	**2.0**	**0.23**	**<0.001**	**1.53 - 2.43**
Q17	**1.5**	**0.23**	**<0.001**	**1.04 - 1,95**	**1.4**	**0.22**	**<0.001**	**0.94 - 1.81**	**1.4**	**0.24**	**<0.001**	**0.96 - 1.91**	**1.3**	**0.22**	**<0.001**	**0.84 - 1.74**
Q18	**0.9**	**0.23**	**<0.001**	**0.46 - 1,39**	**1.0**	**0.23**	**<0.001**	**0.54 - 1.45**	**1.1**	**0.25**	**<0.001**	**0.63 - 1.62**	**0.9**	**0.23**	**<0.001**	**0.46 - 1.38**
Q19	**1.2**	**0.28**	**<0.001**	**0.59 - 1,72**	**1.1**	**0.27**	**<0.001**	**0.59 - 1.68**	**1.3**	**0.29**	**<0.001**	**0.77 - 1.92**	**1.0**	**0.28**	**0.001**	**0.45 - 1.59**
Q20	**1.3**	**0.27**	**<0.001**	**0.74 - 1,81**	**1.5**	**0.26**	**<0.001**	**1.03 - 2.07**	**1.5**	**0.28**	**<0.001**	**0.97 - 2.09**	**1.5**	**0.27**	**<0.001**	**0.95 - 2.03**

Statistical analysis of questions in table 2 with MMRM for patients who completed the study-specific questionnaire at baseline and after two years (n=38). Estimated mean change (ECh) from baseline to follow-up. Significant level was set on p≤0.05 with a confidence interval (CI) on 95%. Significant items are bold. SE=Standard error. Radiotherapy 2 years follow-up=irradiated patients only. No additional surgery 2 years follow-up=patients that had not undergone major additional ipsilateral surgical procedures



	Q5	Q6	Q7	Q8
Ordinal Trend	1.47	**22.47**	**7.90**	**10.88**
p	0.295	**0.002**	**0.003**	**0.017**
CI 95%	0.72-3.02	**3.10-162.98**	**1.98-31.51**	**1.54-77.09**

Figure 3. Q5-Q8 focusing on sexuality and willingness to be naked. The graphs show the percentage of answers in each category, for each item, at baseline and after two years. The ordinal trend is the probability of the patient grading an item one category more positively after two years than at baseline. Significant items are given in boldface. CI=confidence interval



	PF	RP	BP	GH	VT	SF	RE	MH
Reference population	83,1	80,1	70,0	72,8	67,9	87,5	84,6	79,9
Baseline	88,6	85,5	79,9	76,8	67,4	91,8	89,2	80,1
2 years	87,9	86,8	79,6	74,1	67,5	92,8	93,9	82,4
Baseline - Reference population	5,5	5,4	9,9	3,9	-0,5	4,3	4,6	0,2
2 years - Reference population	4,8	6,7	9,6	1,3	-0,4	5,3	9,4	2,5
Baseline - 2 years	0,7	-1,3	0,3	2,6	-0,1	-1,0	-4,8	-2,3
Detectable point differences	10	20	10	10	10	10	20	10

Figure 4. Results for the eight domains of SF-36 of the study population and the age matched reference population of Swedish women (n=3260). PF=physical functioning, RP=physical role functioning, BP=bodily pain, GH=general health, VT= vitality, SF=social role functioning, RE=emotional role functioning, MH=mental health.

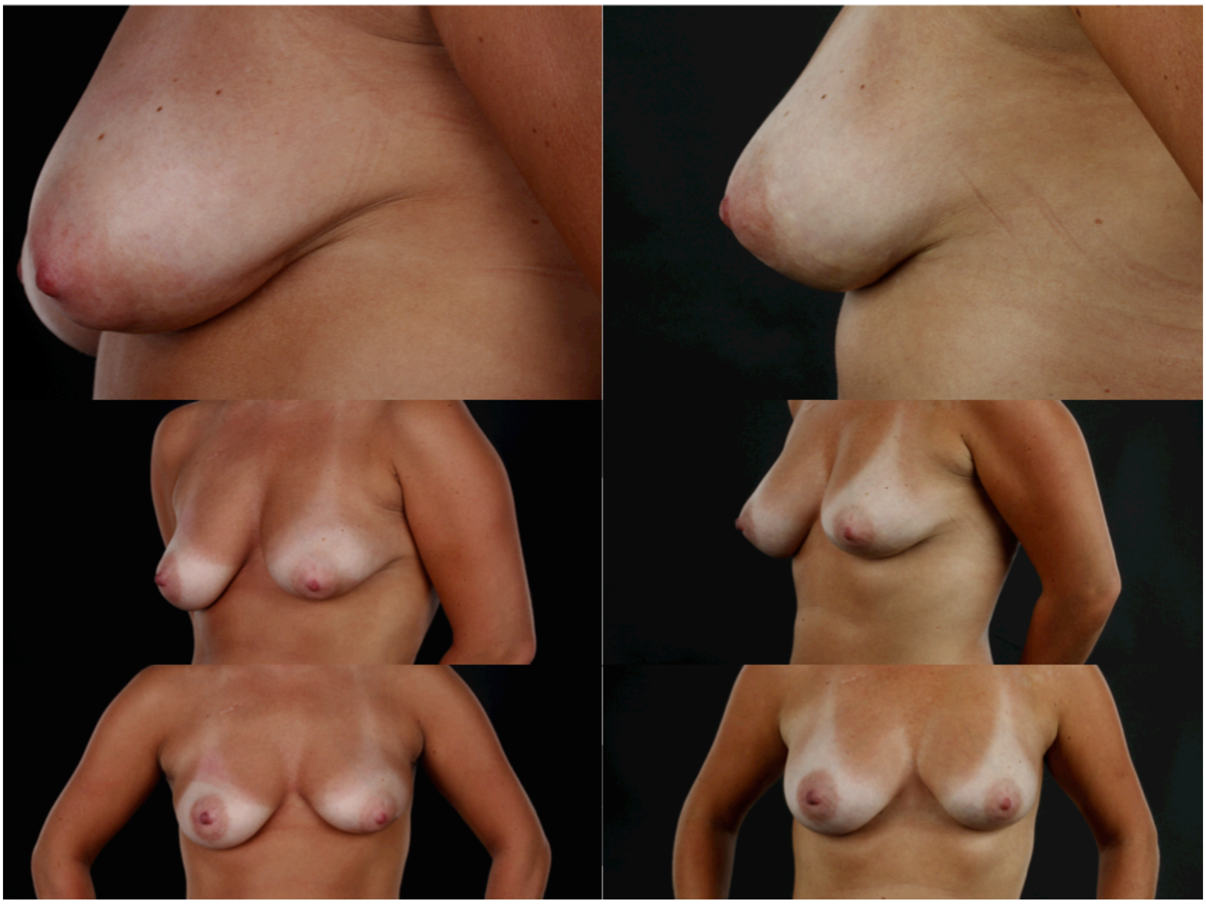


Figure 5. A 31-year-old women that presented with a scar retraction and volume deficiency 3.6 years after breast conserving surgery and radiotherapy (left). She underwent three AFT procedures with totally 150 ml fat. 2.2 years post-operative the scar is not visible and the symmetry is better (right).

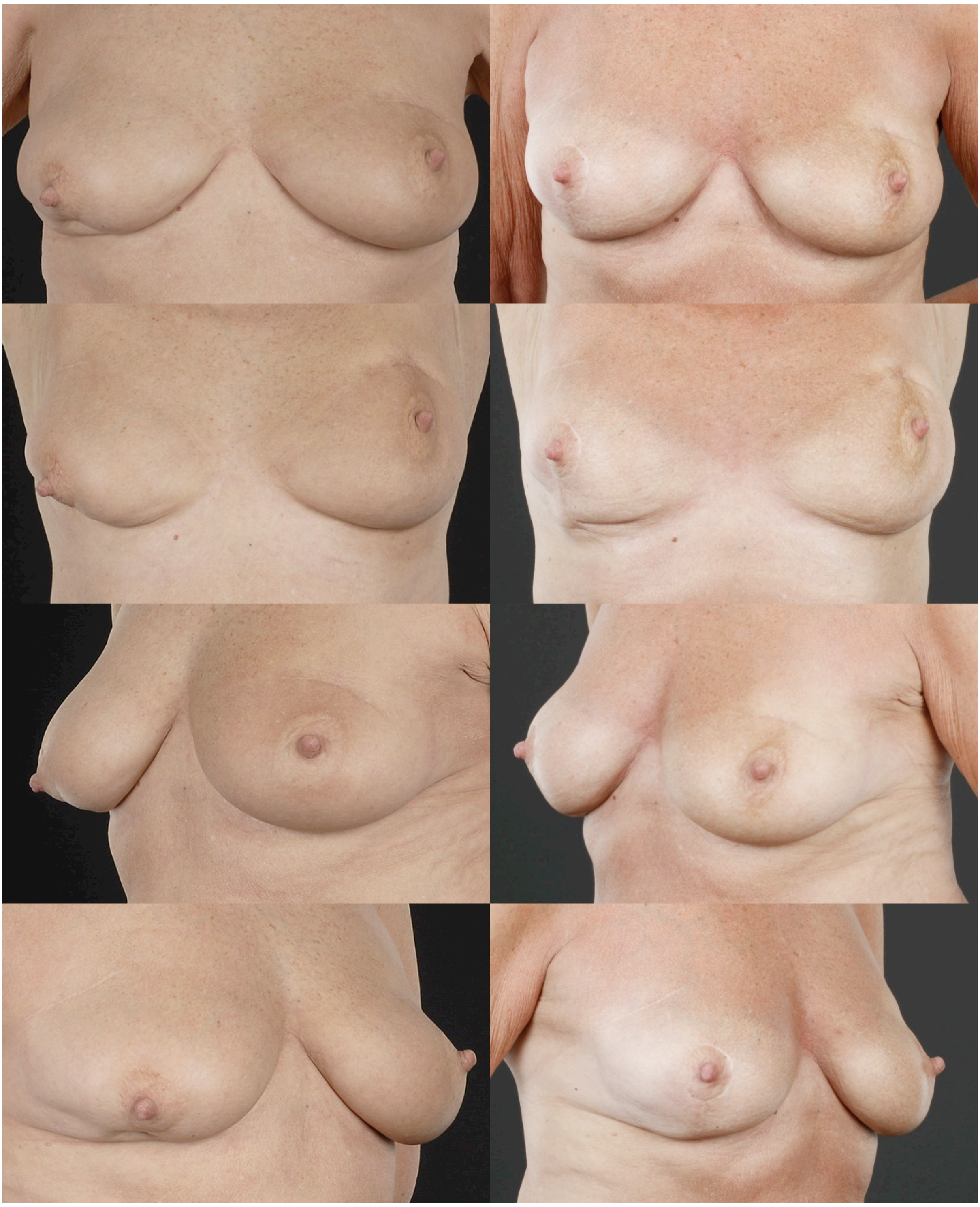


Figure 6. A 66-year-old woman with bilateral defects after BCS and RT (left). Time from RT to AFT was 2.6 years for the left breast and 1 year for the right breast. She underwent two AFT procedures to each breast with totally 69 ml to the left side and 100 ml to the right. In addition she underwent mastopexy on the right side. Post-operative images from the two-year follow-up are shown to the right.

References

1. Elder EE, Brandberg Y, Bjorklund T, Rylander R, Lagergren J, Jurell G, et al. Quality of life and patient satisfaction in breast cancer patients after immediate breast reconstruction: a prospective study. Breast (Edinburgh, Scotland). 2005;14(3):201-8. Epub 2005/06/02.
2. Eltahir Y, Werners LL, Dreise MM, van Emmichoven IA, Jansen L, Werker PM, et al. Quality-of-life outcomes between mastectomy alone and breast reconstruction: comparison of patient-reported BREAST-Q and other health-related quality-of-life measures. Plastic and Reconstructive Surgery. 2013;132(2):201e-9e. Epub 2013/07/31.
3. Rowland JH, Holland JC, Chaglassian T, Kinne D. Psychological response to breast reconstruction. Expectations for and impact on postmastectomy functioning. Psychosomatics. 1993;34(3):241-50. Epub 1993/05/01.
4. Barry M, Kell MR. Radiotherapy and breast reconstruction: a meta-analysis. Breast cancer research and treatment. 2011;127(1):15-22. Epub 2011/02/22.
5. Kling RE, Mehrara BJ, Pusic AL, Young VL, Hume KM, Crotty CA, et al. Trends in autologous fat grafting to the breast: a national survey of the american society of plastic surgeons. Plastic and Reconstructive Surgery. 2013;132(1):35-46. Epub 2013/06/29.
6. de Blacam C, Momoh AO, Colakoglu S, Tobias AM, Lee BT. Evaluation of clinical outcomes and aesthetic results after autologous fat grafting for contour deformities of the reconstructed breast. Plastic and Reconstructive Surgery. 2011;128(5):411e-8e. Epub 2011/10/28.
7. Kanchwala SK, Glatt BS, Conant EF, Bucky LP. Autologous fat grafting to the reconstructed breast: the management of acquired contour deformities. Plastic and Reconstructive Surgery. 2009;124(2):409-18. Epub 2009/08/01.
8. Groen JW, Negenborn VL, Twisk DJ, Rizopoulos D, Ket JC, Smit JM, et al. Autologous fat grafting in onco-plastic breast reconstruction: A systematic review on oncological and radiological safety, complications, volume retention and patient/surgeon satisfaction. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2016;69(6):742-64. Epub 2016/04/18.
9. Delay E, Garson S, Tousson G, Sinna R. Fat Injection to the Breast: Technique, Results, and Indications Based on 880 Procedures Over 10 Years. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 2009;29(5):360-76. Epub 2009/10/15.
10. Lindegren A, Halle M, Docherty Skogh AC, Edsander-Nord A. Postmastectomy breast reconstruction in the irradiated breast: a comparative study of DIEP and latissimus dorsi flap outcome. Plastic and Reconstructive Surgery. 2012;130(1):10-8. Epub 2012/06/30.

11. Beesley H, Ullmer H, Holcombe C, Salmon P. How patients evaluate breast reconstruction after mastectomy, and why their evaluation often differs from that of their clinicians. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2012;65(8):1064-71. Epub 2012/04/06.
12. Kim MS, Sbalchiero JC, Reece GP, Miller MJ, Beahm EK, Markey MK. Assessment of breast aesthetics. Plastic and Reconstructive Surgery. 2008;121(4):186e-94e. Epub 2008/03/20.
13. Wachter T, Edlinger M, Foerg C, Djedovic G, Mayerl C, Kinzl J, et al. Differences between patients and medical professionals in the evaluation of aesthetic outcome following breast reconstruction with implants. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2014;67(8):1111-7. Epub 2014/05/27.
14. Black N. Patient reported outcome measures could help transform healthcare. BMJ (Clinical research ed). 2013;346:f167. Epub 2013/01/30.
15. Ishaque S, Karnon J, Chen G, Nair R, Salter AB. A systematic review of randomised controlled trials evaluating the use of patient-reported outcome measures (PROMs). Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2018. Epub 2018/10/05.
16. Kalaaji A, Bruheim M. Quality of life after breast reconstruction: comparison of three methods. Scandinavian journal of plastic and reconstructive surgery and hand surgery / Nordisk plastikkirurgisk forening [and] Nordisk klubb for handkirurgi. 2010;44(3):140-5. Epub 2010/05/13.
17. Largo RD, Tchang LA, Mele V, Scherberich A, Harder Y, Wettstein R, et al. Efficacy, safety and complications of autologous fat grafting to healthy breast tissue: a systematic review. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2014;67(4):437-48. Epub 2014/01/08.
18. Schultz I, Lindegren A, Wickman M. Improved shape and consistency after lipofilling of the breast: patients' evaluation of the outcome. Journal of plastic surgery and hand surgery. 2012;46(2):85-90. Epub 2012/04/05.
19. Coleman SR. Structural fat grafting. Aesthetic surgery journal / the American Society for Aesthetic Plastic surgery. 1998;18(5):386, 8. Epub 1998/09/01.
20. Brandberg Y, Malm M, Blomqvist L. A prospective and randomized study, "SVEA," comparing effects of three methods for delayed breast reconstruction on quality of life, patient-defined problem areas of life, and cosmetic result. Plastic and Reconstructive Surgery. 2000;105(1):66-74; discussion 5-6. Epub 2000/01/08.

21. Edsander-Nord A, Brandberg Y, Wickman M. Quality of life, patients' satisfaction, and aesthetic outcome after pedicled or free TRAM flap breast surgery. Plastic and Reconstructive Surgery. 2001;107(5):1142-53; discussion 54-5. Epub 2001/05/25.

22. Gahm J, Jurell G, Edsander-Nord A, Wickman M. Patient satisfaction with aesthetic outcome after bilateral prophylactic mastectomy and immediate reconstruction with implants. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2010;63(2):332-8. Epub 2008/12/17.

23. Sullivan M, Karlsson J, Ware JE, Jr. The Swedish SF-36 Health Survey--I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. Social science & medicine (1982). 1995;41(10):1349-58. Epub 1995/11/01.

24. Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plastic and Reconstructive Surgery. 2007;119(5):1409-22; discussion 23-4. Epub 2007/04/07.

25. Agresti A, Kateri M. Categorical Data Analysis. In: Lovric M, editor. International Encyclopedia of Statistical Science. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 206-8.

26. Agha RA, Fowler AJ, Pidgeon TE, Wellstead G, Orgill DP. The Need for Core Outcome Reporting in Autologous Fat Grafting for Breast Reconstruction. Annals of plastic surgery. 2016;77(5):506-12. Epub 2015/11/07.

27. Agha RA, Pidgeon TE, Borrelli MR, Dowlut N, Orkar TK, Ahmed M, et al. Validated Outcomes in the Grafting of Autologous Fat to the Breast: The VOGUE Study. Development of a Core Outcome Set for Research and Audit. Plastic and Reconstructive Surgery. 2018;141(5):633e-8e. Epub 2018/04/27.

28. Pearl RA, Leedham SJ, Pacifico MD. The safety of autologous fat transfer in breast cancer: lessons from stem cell biology. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2012;65(3):283-8. Epub 2011/08/09.

29. Klinger M, Caviggioli F, Klinger FM, Giannasi S, Bandi V, Banzatti B, et al. Autologous fat graft in scar treatment. The Journal of craniofacial surgery. 2013;24(5):1610-5. Epub 2013/09/17.

30. Bonomi R, Betal D, Rapisarda IF, Kalra L, Sajid MS, Johri A. Role of lipomodelling in improving aesthetic outcomes in patients undergoing immediate and delayed

reconstructive breast surgery. European Journal of Surgical Oncology. 2013;39(10):1039-45. Epub 2013/07/31.

31. Sinna R, Delay E, Garson S, Delaporte T, Toussoun G. Breast fat grafting (lipomodelling) after extended latissimus dorsi flap breast reconstruction: a preliminary report of 200 consecutive cases. Journal of plastic reconstructive & aesthetic surgery : JPRAS. 2010;63(11):1769-77. Epub 2010/01/19.

32. Bennett KG, Qi J, Kim HM, Hamill JB, Wilkins EG, Mehrara BJ, et al. Association of Fat Grafting With Patient-Reported Outcomes in Postmastectomy Breast Reconstruction. JAMA surgery. 2017;152(10):944-50. Epub 2017/06/29.

33. Pelusi J. Sexuality and body image. Research on breast cancer survivors documents altered body image and sexuality. The American journal of nursing. 2006;106(3 Suppl):32-8. Epub 2006/02/17.

34. Costantino A, Fioramonti P, Ciotti M, Onesti MG. Lipofilling in skin affected by radiodermatitis: clinical and ultrasound aspects. Case report. Il Giornale di chirurgia. 2012;33(5):186-90. Epub 2012/06/20.

35. Garza RM, Paik KJ, Chung MT, Duscher D, Gurtner GC, Longaker MT, et al. Studies in fat grafting: Part III. Fat grafting irradiated tissue--improved skin quality and decreased fat graft retention. Plastic and Reconstructive Surgery. 2014;134(2):249-57. Epub 2014/07/30.

36. Mojallal A, Lequeux C, Shipkov C, Breton P, Foyatier JL, Braye F, et al. Improvement of skin quality after fat grafting: clinical observation and an animal study. Plastic and Reconstructive Surgery. 2009;124(3):765-74.

37. Kumar R, Griffin M, Adigbli G, Kalavrezos N, Butler PE. Lipotransfer for radiation-induced skin fibrosis. The British journal of surgery. 2016;103(8):950-61. Epub 2016/05/14.

38. Panettiere P, Marchetti L, Accorsi D. The serial free fat transfer in irradiated prosthetic breast reconstructions. Aesthetic Plast Surg. 2009;33(5):695-700. Epub 2009/06/02.

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ORIGINAL SCIENTIFIC REPORT

Autologous Fat Transplantation to the Reconstructed Breast Does not Hinder Assessment of Mammography and Ultrasound: A Cohort Study

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Abstract

Background Autologous fat transplantation (AFT) to the breast can correct defects and be a part of a breast reconstruction to achieve a better aesthetic result. The impact of AFT on the radiological evaluation and detection of cancer remains unclarified. The aim of this study is to investigate whether AFT induces lasting modifications.

Methods In the present study, a valuation was performed of 44 breasts from 37 patients examined with mammography and ultrasound before and after autologous fat transplantation. Breast radiologists evaluated the images using a study specific protocol.

Results AFT did not hinder post-operative assessment of mammograms or ultrasound. No detectable changes with serious clinical impact were found after injections of mean 177 ml (34–516) of fat in one to four sessions. The rate of oil cysts was significantly higher after AFT than pre-operatively (2.3 vs. 34.1 % $p = 0.0013$). Significantly more post-operative oil cysts were detected after injection of larger volumes of fat (144 vs. 243 ml, $p = 0.013$). No significant differences were found in the post-operative images regarding age at surgery, follow-up time, or time from previous breast surgery.

Conclusion AFT does not impair assessment of mammograms and ultrasound in patients who have a history of breast cancer surgery or prophylactic mastectomy.

Introduction

Autologous fat transplantation (AFT) to the breast is a frequently used technique for the correction of breast defects and after breast cancer surgery [1–5]. In 1987, the American Society of Plastic and Reconstructive Surgery (ASAP) [6] declared that breast cancer surveillance and screening "will become difficult" when using autologous fat injection to the breast. They also stated that AFT "can inhibit early detection of breast carcinoma and is therefore hazardous to public health." Despite this, AFT has gained in popularity and many reports have been published on standardized techniques with good results [3, 4, 7–11]. In 2009, ASAP published a report from their Fat Graft Task Force [12], recommending that caution be taken regarding "those with risk factors for breast cancer" and that there

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Table 1 Patient characteristics

	n (%)	SD	Min	Max
Patients ($n = 37$)				
Age at first session (Mean)	54.9	8.5	39.1	71.4
Follow-up time (month) (Mean)	15.9	5.3	10.0	28.0
Patients with bilateral lipofilling	7 (18.4)			
Ipsilateral surgery breasts/sessions	7 (18.4)/11 (9.2)			
Capsulotomy + implant replacement	2			
Capsulotomy + implant replacement + scar excision	1			
Liposuction	1			
Liposuction + nipple reconstruction	1			
Nipple correction	1			
Nipple reconstruction	2			
Scar excision	3			
Breasts ($n = 44$)				
Age at first session (mean)	54.9	8.4	39.1	71.4
Follow-up time month (mean)	15.6	5.2	10.0	28.0
Time from last breast surgery to lipofilling month (mean)	47.7	34.4	10.0	152.0
Fat injected ml (mean)	177.4	116.0	34.0	516.0
Previous breast surgery				
Breast conserving surgery	20 (45.5)			
Mastectomy + immediate reconstruction	12 (27.3)			
Mastectomy + delayed reconstruction	1 (2.3)			
Prophylactic mastectomy + immediate reconstruction	7 (15.9)			
Mastectomy + latissimus dorsi flap	4 (9.1)			
Radiotherapy	30 (68.2)			
Number of lipofilling sessions				
1	4 (9.1)			
2	11 (25)			
3	26 (59.1)			
4	3 (6.8)			
Tot	116			
Ultrasound pre-operatively	44 (100)			
Ultrasound post-operatively	44 (100)			
Mammography pre-operatively	29 (65.9)			
Mammography post-operatively	26 (59.1)			
FNAC[a]	9 (20.5)			
Palpable	6 (13.6)			
Visible in ultrasound	3 (6.8)			
Analyzed by cytologist	8 (18.2)			

[a] *FNAC* fine-needle aspiration cytology

might be an interference with "physical examination or breast cancer detection." They concluded that AFT does not interfere with radiological examinations in lack of evidence of the opposite.

During recent years new studies with radiological aspects have been published [13–21], most of these being case reports or case series and only a few including breast cancer patients. Regarding cancer relapse and radiological findings, most studies do not indicate that AFT impairs radiological evaluation but there are still doubts regarding safety in patients with a history of cancer.

The aim of this prospective cohort study was to evaluate radiological and ultrasound-detected changes in the breast after AFT and to further clarify if AFT could hinder future radiological evaluations of the breast or interfere with the detection of cancer. Our hypothesis was that AFT does not complicate the assessment of mammograms and ultrasound for the experienced radiologist in patients who have a history of breast cancer surgery or prophylactic mastectomy and who have undergone AFT.

Materials and methods

This is a prospective over-time cohort study comparing mammography and ultrasound before and after AFT of the breast.

Patients

Thirty-seven consecutive patients (44 breasts) aged 26–75 years of age were enrolled between October 2008 and May 2012. Previous breast surgery is listed in Table 1. Exclusion criteria were <1 year since cancer surgery or radiotherapy, systemic disease such as diabetes mellitus, breast cancer recurrence, or less than 10 months from last AFT to radiological examination. All patients underwent clinical follow-up at 6, 12 and 24 months after the last AFT. At the 12-month follow-up patients underwent mammography and ultrasound.

Surgery

Fat was harvested by dry technique using a Coleman cannula on a 10 ml syringe and centrifuged (3000 rpm, 3 min). The purified lipocytes were separated from liquids and injected fan-shaped in multiple layers into the subcutaneous tissues using another blunt cannula (1.29 mm diameter). All sessions were performed under general anesthesia. One dose of intravenous antibiotics was administered pre-operatively to patients with implants. The patients underwent 1–4 AFT procedures until a satisfactory result was obtained. Seven patients (11 procedures) underwent ipsilateral surgery during one or several operations (Table 1).

Radiology

Pre-operative mammography and ultrasound was performed a maximum of 3 months prior to AFT as a baseline control. Mammograms were obtained using a Giotto mammograph (IMS, Bologna, Italy). When possible, both medio-lateral oblique and cranio-caudal projections were taken. In some patients who had undergone mastectomy, only one projection was taken. Ultrasound was performed using an Acuson Antares Echograph (Siemens Healthcare, Erlangen, Germany) equipped with a 5–13 MHz linear transducer. Two experienced breast radiologists independently assessed all the images. They made the following evaluations compared with the pre-operative assessment: new calcifications or a change in their quantity (together referred to as progress in calcification), oil cysts, fat necrosis, abnormalities or malignancies, and change in BI-RADS (Breast Imaging Reporting and Data System) [22] score and scarring. The BI-RADS scoring system was adopted for the assessment of imaging findings. Individual breast densities were not recorded as AFT did not disturb assessment of the mammograms. When relevant, radiologists or pathologists performed fine-needle aspiration cytology (FNAC) and a cytologist analyzed the tissue.

Ethical approval

Informed consent was obtained, and the study was approved by the Regional Ethics Review Board in Stockholm (2008/484-31/2).

Statistical methods

All analyses were carried out using STATA 13.0. Distributions in continuous variables were tested with the Mann–Whitney test. Differences for categorical data were tested using the chi-square test of independence. Risk ratios are presented together with 95 % confidence intervals.

In the descriptive data, all breasts are included. To be able to analyze data on an individual instead of a breast level [i.e., to eliminate bilateral AFT as a confounder], we excluded one breast in each patient with bilateral AFT. Four of the patients with bilateral AFT had unilateral cancer, and the non-cancer side was excluded. Two patients had bilateral cancer, and we subsequently drew

Table 2 Rates of findings in mammography and ultrasound

	N[a]	%
BI-RADS 2 pre-operative	44	100
BI-RADS 2 post-operative	44	100
Calcifications pre-operative	12	41.4
Calcifications post-operative	17	65.4
Progress of calcification	8	32.0
Oil cysts pre-operative	1	2.3
Oil cysts post-operative	15[b]	34.1
Liponecrosis post-operative	0	0
Scarring post-operative	24	100
Better	8	33.3
Unchanged	16	66.7

[a] Breasts

[b] Difference pre and post-operatively when adjusting for bilateral AFT $p = 0.0013$ McNemar's chi-square

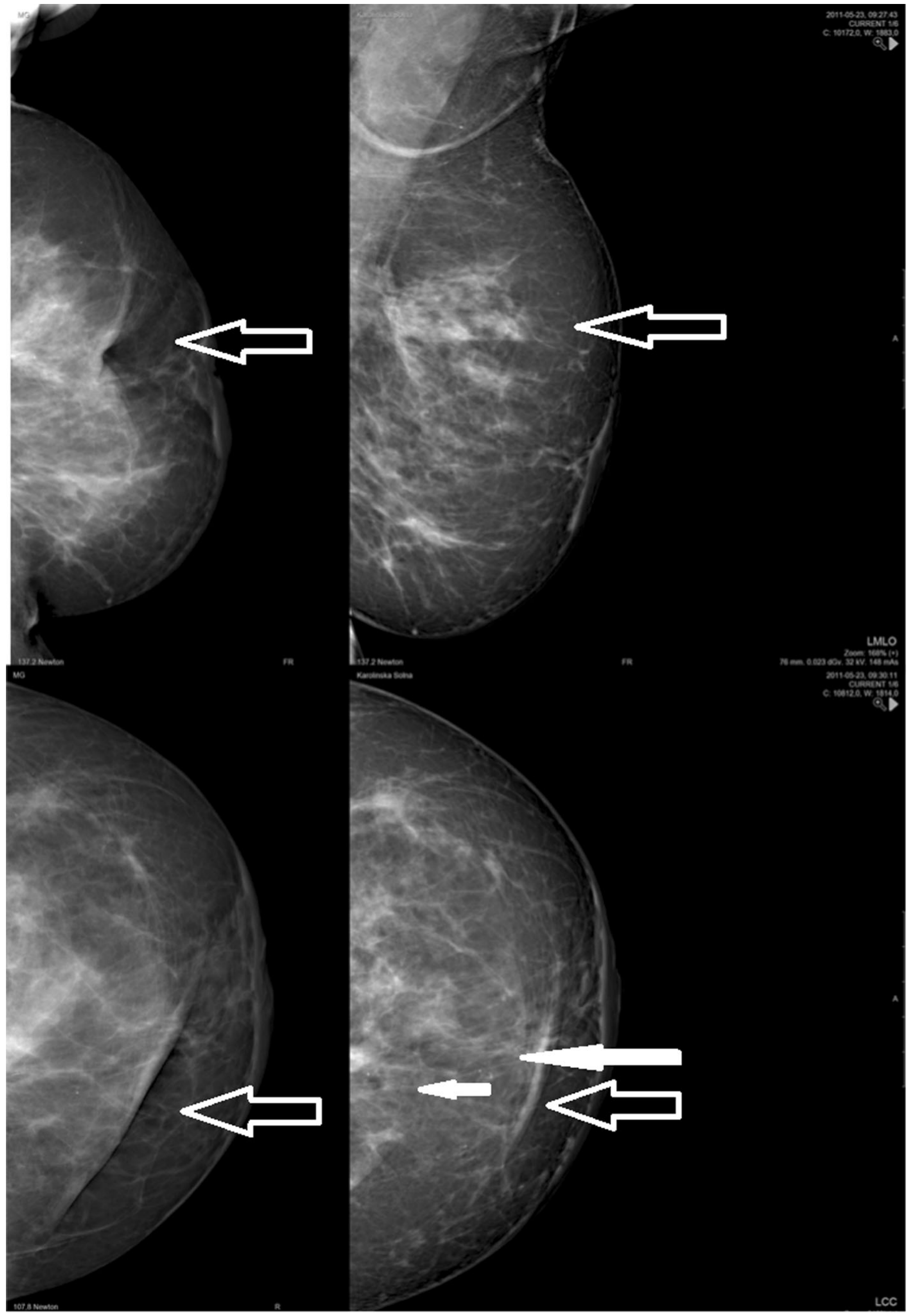

Fig. 1 Mammogram before (*left*) and 14 months after last (*right*) AFT. A 68-year-old woman who underwent two AFTs with a total amount of 115 ml fat injected to her left breast. *White arrows* indicate calcifications. *Transparent arrows* indicate scarring. Left medio-lateral oblique and left cranio-caudal projections

lots. One patient had undergone a bilateral prophylactic mastectomy, and all parameters were identical, and one of the sides was excluded.

Results

The mean age of the patients was 54 (range 39–71) years at follow-up. No patients were lost to follow-up. Mean time from last breast surgery to AFT was 47.7 months (range 10–152). The mean volume of fat injected was 177.4 ml (range 34–516). The mean follow-up time was 15.6 months (range 10–28). All breasts were evaluated pre- and post-operatively with ultrasound. Twenty-nine breasts had mammograms taken pre- and 26 post-operatively. In some patients, it was impossible to perform mammography due to their breast reconstructions (Table 1).

Two experienced radiologists had no difficulties interpreting the mammograms and ultrasounds after AFT. All patients had a BI-RADS score 2 (=benign findings) pre- and post-operatively (Table 2). No patients had any abnormalities or malignancies.

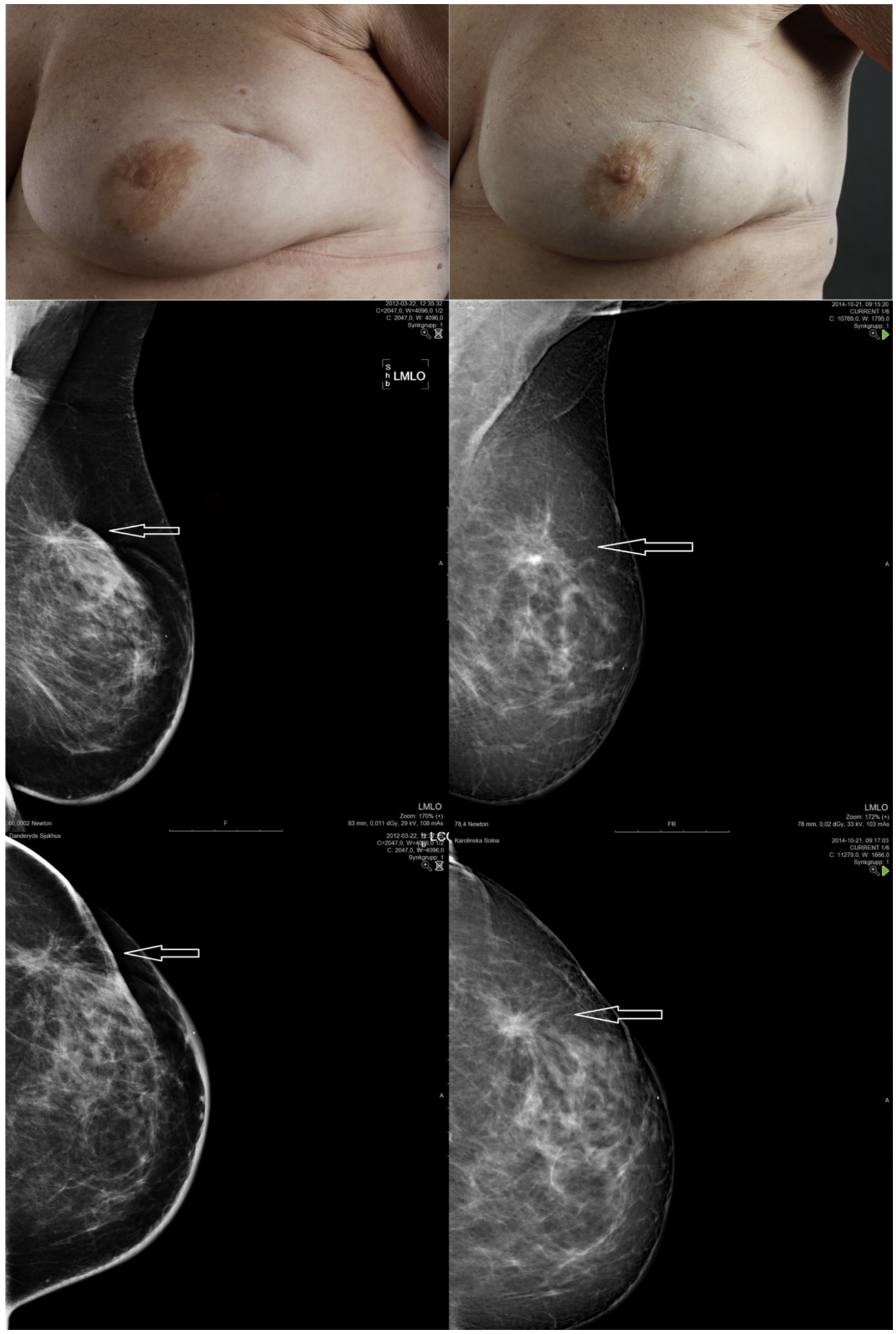

Fig. 2 Photograph and mammogram before (*left*) and 13 months after last AFT (*right*). A 61-year-old woman who underwent three AFTs with a total amount of 268 ml fat injected to her left breast. *Arrows* indicate scarring. Left medio-lateral oblique and left cranio-caudal projections

Twelve (41.4 %) breasts had calcifications pre- and 17 (65.4 %) post-operatively (Figs. 1, 2). Four of the 12 with pre-operative calcifications had an increased amount of calcifications, and, totally, 8 out of 25 (32 %) patients had progress in calcification. All calcifications were clinically irrelevant however, and would not have been reported by the radiologist in clinical practice. There was no significant difference in progress between those with and without pre-operative calcifications ($p = 0.879$, RR = 1.1, 95 % CI 0.36–3.34) (Table 3). There were no differences in mean time to follow-up, volume injected fat, age at surgery, or time from last surgery to AFT between those with progress in calcification at follow-up and those without progress (Table 4). Pre-operative radiotherapy did not significantly affect progress in calcification.

One patient had an oil cyst pre-operatively. Post-operatively, 15 (34.1 %) breasts had oil cysts detected with ultrasound ($p = 0.0013$) (Fig. 3). In these breasts, a

Table 3 Rate ratio for progress of calcifications comparing with or without pre-operative calcification

	Progress of calcifications		
	No	Yes	Total
Pre-operative calcifications			
No	8	4	12
Yes	7	4	11
Total	15	8	23

$p = 0.8789$ RR = 1.09 CI 0.36–3.34

significantly higher amount of fat had been injected ($p = 0.013$), but there was no difference in follow-up time, age, or time from prior surgery. No fat necrosis could be found (Table 2).

Scarring was detected by mammography in all patients pre-operatively (Figs. 1, 2). Twenty-four had both pre and post-operative mammography done. Eight (33.3 %) of these had regress of scarring after AFT with smoother breast contours (Table 5, Fig. 2). When adjusting for bilateral AFT, there were no differences in mean time to follow-up, fat volume, age, or time from last surgery to AFT between those with and without regress of scarring (Table 4).

At follow-up after AFT, six out of 44 patients had a palpable lump that was further examined with FNAC. Additionally, three breasts (6.8 %) had FNAC initiated by the radiologist because of ultrasound findings. One of the aspirations consisted of oil only and was discarded. Histopathology showed fat necrosis in four breasts, benign breast tissue, and oil in two breasts, one breast with normal fat tissue and one cyst in one breast (Table 1).

Table 4 Progress in calcifications and scarring in mammography and in oil cysts in ultrasound

	Median	Min	Max	p^a
Follow-up (months)				
Progress −	15.00	11.00	27.00	0.28
Progress +	13.50	10.00	23.00	
Amount of fat injected				
Progress −	165.5	40.00	322.00	0.12
Progress +	233.0	115.00	445.00	
Age at session 1				
Progress −	54.87	39.08	66.61	0.95
Progress +	53.13	42.82	71.37	
Time from last surgery to lipofilling				
Progress −	34.00	21.00	98.00	0.37
Progress +	45.50	20.00	120.00	
Follow-up (months)				
Oil cysts −	13.00	10.00	28.00	0.70
Oil cysts +	13.00	10.00	27.00	
Amount of fat injected				
Oil cysts −	144.00	40.00	419.00	**0.013**
Oil cysts +	243.00	74.50	516.00	
Age at session 1				
Oil cysts −	56.20	39.08	71.37	0.80
Oil cysts +	49.41	44.79	67.59	
Time from last surgery to lipofilling				
Oil cysts −	30.00	10.00	152.00	0.36
Oil cysts +	39.00	20.00	120.00	
Follow-up (months)				
Scarring better	13.00	10.00	23.00	0.17
Scarring unchanged	14.50	11.00	27.00	
Amount of fat injected				
Scarring better	231.00	115.00	445.00	0.39
Scarring unchanged	170.00	40.00	322.00	
Age at session 1				
Scarring better	57.59	46.76	67.59	0.46
Scarring unchanged	54.63	39.08	71.37	
Time from last surgery to lipofilling				
Scarring better	28.00	20.00	120.00	0.84
Scarring unchanged	38.50	21.00	98.00	

Bold value indicates significant p-value

[a] Mann–Whitney test

Discussion

The most important issue regarding AFT is that of oncological safety. This is not only a matter of possible AFT-induced relapse or new cancers but also the possibility of detecting malignancies. In the oncological follow-up, it is important that the surgeon, the oncologist, and the radiologist feel familiar with the reconstruction method and the possibility of examining a breast after AFT. Krastev et al. recently published a systematic review [18] on the oncological safety of AFT and reported, with few exceptions, small studies, short follow-up times, and low evidence levels. Conclusions cannot be drawn even from larger studies [23–25] due to incomparable patient groups and diverging results with significant overlap in the patient groups [23, 24].

Wang [15] reported a 16.7 % frequency of “highly suspicious” clustered microcalcifications after breast augmentation with fat transplantation. Rietjens et al. [13] reported 6.5 % calcifications in their control mammograms. In Claro et al’s review [26], which is a mix of studies with aesthetic and reconstructive focus with mainly aesthetic patients, 1.6 % of the patients had microcalcifications and 8.7 % had cysts. Kim et al. [19] recently reported a 17.6 % frequency in fat necrosis and cysts but did not report any information on calcifications. The high frequency of calcifications (65.4 %) in our patients compared with other reports is probably due to our radiologists

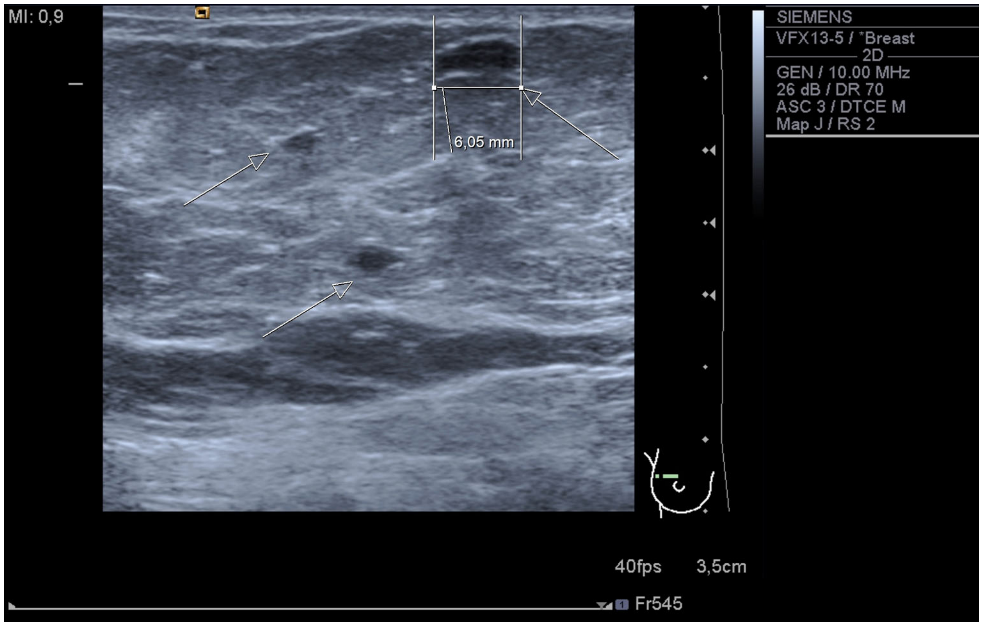

Fig. 3 Ultrasound image after AFT, *arrows* indicate oil cysts

recording even minimal calcifications. In clinical practice, these minimal calcifications and progresses would probably not be mentioned in the radiological statements since they are of no clinical interest. No significant difference in volume between those with or without progress was found. A tendency could be observed that the breasts with progress in calcifications had 46 % (244.2 vs.181.9) more fat injected into them. This might imply that a larger amount of fat generates more calcifications, a finding that is also supported by Wang [27].

In a Japanese study [28], several patients who had previously undergone augmentation with large amounts of autologous fat presented with large masses of fat necrosis and oil cysts that had to be removed surgically. Even with the injection of smaller amounts of fat, as in the present study, AFT induced oil cysts and their presence depended on the volume of fat injected. This emphasizes the importance of good surgical technique especially when larger amounts of fat are injected. It also raises the question of how large a volume of fat is reasonable to inject at one time or if smaller volumes in several sessions are preferable.

Recently, Agha et al. [29] published a systematic review on autologous fat grafting, including 17 studies regarding radiology. As in the present study, they exclusively included studies on reconstructive breast surgery. They reported a 2.7 % frequency of radiological abnormalities that had to be further examined by biopsy. We had a higher frequency (6.8 %). Eighty percent of the reviewed studies were case reports or series and, therefore, hard to draw conclusions from.

Patients with regress in scarring (33 %) had had 34 % more fat injected than those with unchanged scarring. This difference was not significant, perhaps due to the relatively small number of patients included. The result of fat grafting can be improved in many patients by multiple sessions of AFT, instead of overfilling in one session.

As AFT has become a common and important complement in breast reconstructions, giving the reconstructive surgeon further possibilities to achieve a good final result, not only radiologists at centers where the method is well known will assess mammograms and perform ultrasound on these women. The findings in the present study, that evaluation of mammography and ultrasound was not impaired, is in accordance with other studies [14] and can facilitate the follow-up of these women also in other settings than breast centers. Our study indicates that if a patient undergoes AFT using modern techniques, there is no risk of interference with future follow-up using mammography and ultrasound.

A strength of our study is that we only included patients with former breast cancer and those at high risk of developing the disease, patients operated with prophylactic surgery, and no aesthetic patients. A limitation is that some of the patients with prosthesis could not have mammography performed, but only ultrasound.

We did not include MRI because it is not used for the standard evaluation of breast cancer patients in Sweden according to the Swedish National Guidelines [30].

The patients in this study underwent treatment with relatively small amounts of fat. Hence, the results can only be generalized to similar patient groups. In conclusion, no detectable serious negative effects were seen after AFT using relatively small amounts of fat to the breast in

Table 5 Post-operative scarring compared to pre-operatively

Scarring	*n*	%
Better	8	33.33
Unchanged	16	66.67

women with previous breast cancer surgery or prophylactic mastectomy and reconstruction. AFT did not hinder the assessment of mammograms or ultrasound. We found a significantly increased number of oil cysts after injection of larger volumes.

This study supports the safety of AFT in the aspect of radiological follow-up of breast cancer and prophylactic patients.

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Compliance with Ethical Standards

Conflicts of interest None. The sponsors have no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

References

1. Spear SL, Wilson HB, Lockwood MD (2005) Fat injection to correct contour deformities in the reconstructed breast. Plast Reconstr Surg 116(1300–130):5
2. Kwak JY, Lee SH, Park HL et al (2004) Sonographic findings in complications of cosmetic breast augmentation with autologous fat obtained by liposuction J Clinical. Ultrasound 32(299–30):1
3. Coleman SR, Saboeiro AP (2007) Fat grafting to the breast revisited: safety and efficacy. Plast Reconstr Surg 119:775–785 **(discussion 786–777)**
4. Missana MC, Laurent I, Barreau L et al (2007) Autologous fat transfer in reconstructive breast surgery: indications, technique and results. Eur J Surg Oncol (EJSO) 33:685–690
5. Rigotti G, Marchi A, Galie M et al (2007) Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 119:1409–1422 **(discussion 1423–1404)**
6. ASPRS Ad-Hoc Committee on New Procedures (1987) Report on autologous fat transplantation. Plast Surg Nurs 7:140–141
7. Illouz YG, Sterodimas A (2009) Autologous fat transplantation to the breast: a personal technique with 25 years of experience. Aesthet Plast Surg 33:706–715
8. Kanchwala SK, Glatt BS, Conant EF et al (2009) Autologous fat grafting to the reconstructed breast: the management of acquired contour deformities. Plast Reconstr Surg 124:409–418
9. Khouri R, Del Vecchio D (2009) Breast reconstruction and augmentation using pre-expansion and autologous fat transplantation. Clin Plast Surg 36:269–280
10. Elfadl D, Garimella V, Mahapatra TK et al (2010) Lipomodelling of the breast: a review. Breast 19:202–209
11. Delay E (2010) CHAPTER 12: fat injections to the breast: the lipomodeling technique. In: Hall-Findlay EJ, Evans GRD (eds) Aesthetic and reconstructive surgery of the breast. Saunders, Edinburgh, pp 171–192
12. Gutowski KA (2009) Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. Plast Reconstr Surg 124:272–280
13. Rietjens M, De Lorenzi F, Rossetto F et al (2011) Safety of fat grafting in secondary breast reconstruction after cancer. J Plast Reconstr Aesthet Surg 64:477–483
14. Veber M, Tourasse C, Toussoun G et al (2011) Radiographic findings after breast augmentation by autologous fat transfer. Plast Reconstr Surg 127:1289–1299
15. Wang CF, Zhou Z, Yan YJ et al (2011) Clinical analyses of clustered microcalcifications after autologous fat injection for breast augmentation. Plast Reconstr Surg 127:1669–1673
16. Rubin JP, Coon D, Zuley M et al (2012) Mammographic changes after fat transfer to the breast compared with changes after breast reduction: a blinded study. Plast Reconstr Surg 129:1029–1038
17. Costantini M, Cipriani A, Belli P et al (2013) Radiological findings in mammary autologous fat injections: a multi-technique evaluation. Clin Radiol 68:27–33
18. Krastev TK, Jonasse Y, Kon K (2013) Oncological safety of autologous lipoaspirate grafting in breast cancer patients: a systematic review. Ann Surg Oncol 20:111–119
19. Kim HY, Jung BK, Lew DH et al (2014) Autologous Fat Graft in the Reconstructed Breast: Fat Absorption Rate and Safety based on Sonographic Identification Arch Plast Surg 41:740–747
20. Carvajal J (2008) Patino JH Mammographic findings after breast augmentation with autologous fat injection Aesthet Surg J 28:153–162
21. Brenelli F, Rietjens M, De Lorenzi F et al (2014) Oncological safety of autologous fat grafting after breast conservative treatment: a prospective evaluation. Breast J 20:159–165
22. D'Orsi CJ, Mendelson EB, Ikeda DM et al (2003) Breast imaging reporting and data system: ACR BI-RADS: breast imaging atlas. American College of Radiology, Reston
23. Petit JY, Botteri E, Lohsiriwat V et al (2012) Locoregional recurrence risk after lipofilling in breast cancer patients. Ann Oncol 23:582–588
24. Petit JY, Lohsiriwat V, Clough KB et al (2011) The oncologic outcome and immediate surgical complications of lipofilling in breast cancer patients: a multicenter study–Milan–Paris–Lyon experience of 646 lipofilling procedures. Plast Reconstr Surg 128:341–346
25. Rigotti G, Marchi A, Stringhini P et al (2010) Determining the oncological risk of autologous lipoaspirate grafting for post-mastectomy breast reconstruction. Aesthetic Plast Surg 34:475–480
26. Claro F Jr, Figueiredo JC, Zampar AG et al (2012) Applicability and safety of autologous fat for reconstruction of the breast. Br J Surg 99:768–780
27. Wang H, Jiang Y, Meng H et al (2010) Sonographic identification of complications of cosmetic augmentation with autologous fat obtained by liposuction. Ann Plast Surg 64:385–389
28. Hyakusoku H, Ogawa R, Ono S et al (2009) Complications after autologous fat injection to the breast. Plast Reconstr Surg 123:360–370 **(discussion 371–362)**
29. Agha RA, Fowler AJ, Herlin C et al (2015) Use of autologous fat grafting for breast reconstruction: a systematic review with meta-analysis of oncological outcomes. J Plast Reconstr Aesthet Surg 68:143–161 30.
30. Group SSBC (2013) NATIONELLA RIKTLINJER FÖR BEHANDLING AV BRÖSTCANCER. SweBCG Swedish Breast Cancer Group. http://www.swebcg.se/index.asp?P=NatRikt.

Title
Autologous fat transplantation alters gene expression patterns related to inflammation and hypoxia in the irradiated human breast

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What is already known: Clinical studies indicate that autologous fat transplantation (AFT) stimulates regression of chronic inflammation and fibrosis caused by radiotherapy in skin and subcutaneous fat. However, there is a paucity of biological evidence and the underlying processes are poorly understood. Human data are scarce, whereas experimental animal studies have mainly focused either on the effect of irradiation or AFT alone.

What is new: The current study indicates that radiotherapy causes dysregulated gene expression in fibrosis-related pathways within adipose tissues in humans. It also shows that AFT causes a reversal of this with several dysregulated genes returning to nearly normal expression levels.

Potential impact on future practice: The study provides biological evidence for AFT's impact on radiation-induced dysregulated gene expression in humans. The study supports AFT to be indicated as treatment for radiation induced fibrosis, associated with severe morbidity and surgical challenges.

Abstract

Background
Radiation-induced fibrosis (RIF) is associated with functional and cosmetic impairment as well as surgical complications. Clinical reports suggest improvement following autologous fat transplantation (AFT), but the underlying mechanisms remain unclear. A global gene expression strategy was undertaken to identify how radiotherapy (RT) affected gene expression patterns and furthermore if AFT could affect these patterns.

Method
Adipose tissue biopsies were taken synchronously from irradiated, and contralateral non-irradiated breasts, before and one year after AFT. The 3000 most dysregulated genes, comparing both breasts, were subjected to Hallmark analysis to detect enriched pathways among genes dysregulated by radiation. A significant enrichment of genes related to a specific pathway was defined as $p < 0.05$ and false discovery rate (FDR) < 0.05 was used to correct for type I errors. A similar comparison was conducted post-AFT to study the effect of AFT on gene expression patterns.

Results
Forty microarrays were analysed from biopsies taken from 10 patients pre-, and post-AFT. Forty-five pathways were identified among the 3000 most dysregulated transcripts pre-RT compared to the non-irradiated breast. Post-AFT 575 genes were altered anew and 13 pathways identified ($p<0.05$; FDR<0.05). The top two canonical pathways were interferon gamma response and hypoxia. Correlative immunohistochemistry also indicate increased macrophage recruitment in irradiated tissues.

Conclusion
The study identifies dysregulated gene expression patterns in irradiated human breast adipose tissue and shows that AFT affects several pathways related to fibrosis. It contributes to the understanding of how AFT can ameliorate RIF and brings biological support to AFT as potential treatment against RIF.

Introduction

With on-going improvements in cancer care, a growing population of survivors suffer from the late adverse effects after radiotherapy (RT), such as radiation-induced fibrosis (RIF), resulting in tissue scarring, induration and contracture. Clinical data mainly involve RIF after breast and head-neck cancer, where radiotherapy is commonly used and the impact is paramount on both functional and cosmetic outcome. RIF can adversely impact aesthetic outcomes after breast reconstruction resulting in asymmetry or deformity. Autologous fat transplantation (AFT) has gained significant popularity in the treatment of RIF and has shown, in clinical series, to improve the quality of irradiated skin and augment local volume [1].

Recent studies describe improvements in RIF following AFT [2, 3]. However, biological effects have mainly been studied in animal models[4-6] and are still not fully understood. Efforts to explain radiation-induced fibrosis, for example, by genetic variation have remained largely inconclusive[7]. The authors have previously described global gene expression alterations in irradiated recipient vessels from free flap breast and head neck reconstructions[8, 9], but there is a paucity of studies that have been able to describe gene expression patterns of the irradiated breast adipose tissue. Furthermore, the effect of AFT on gene expression profiles in humans is largely unknown. Therefore, a complete gene expression profiling strategy of unilaterally irradiated breasts has been undertaken and alterations following AFT have been studied, with the non-irradiated breast as a continuous control. Accordingly, we proceeded to identify shared and unique gene expression properties between irradiated and non-irradiated breast adipose tissue before and after AFT in humans.

Material and Methods

Patient selection

From June 2009 to March 2015 breast cancer patients at the Department of Reconstructive Plastic surgery at Karolinska University Hospital in Stockholm, Sweden were invited to participate. Women who had undergone previous breast surgery (breast conservation or mastectomy) and adjuvant radiotherapy, at least 12-months prior to recruitment, and who required AFT were included. Women with breast cancer recurrences and systemic inflammatory diseases were excluded. Informed consent was obtained from 30 patients.

Autologous fat transplantation

Fat was harvested using a dry technique, under general anaesthetic. In two patients tumescent liposuction was performed in one out of three AFT sessions due to a change in choice of method at the unit. The remaining parts of the AFT were performed identically. The fat was injected subcutaneously as previously described[10].

Human sample collection

Synchronous, bilateral excision biopsies of fat tissue were taken from the irradiated and non-irradiated breast from 30 women at two occasions (totally 104 biopsies) by a single surgeon. The preoperative biopsies were taken immediately before AFT and the postoperative biopsy, approximately one year after AFT. Biopsies from the irradiated breast were taken from a location where AFT was planned (ensuring the previous scar was not included) and in the control breast from the lower pole where the small scar would be less visible. Tissues were stored in either RNA-later® (Qiagen, Hilden, Germany) or in liquid nitrogen and stored at -80°C for RNA purification and in formalin for immunohistochemistry. The samples were registered in the Stockholm Medical Biobank (SMB). The study was approved by the Ethical review board in Stockholm (2008/484-31/2).

RNA extraction

Extraction of RNA was performed using the RNeasy Lipid Tissue Mini kit® (Qiagen, Hilden, Germany) as per the manufacturer's protocol. RNA quality was analysed by micro capillary electrophoresis using an Agilent Bioanalyzer® (Agilent, Santa Clara, USA) with RNA 6000 Pico Kit and Agilent 2200 TapeStation with RNA Screen Tape. The amount of RNA was determined by UV spectrophotometry with a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, USA). RNA samples were prepared from total RNA (100ng) for whole transcriptome expression analysis using the WT Plus reagent kit (Affymetrix, Santa Clara, USA). The kit generates amplified and biotinylated sense-strand DNA targets for hybridization to Gene Chip Sense Target Array plates. To be eligible for gene expression analysis (microarray), only samples achieving an RNA integrity number (RIN) of greater than 4.8 with a total available quantity of ≥ 100 ng were selected. This was carried out by the core facility for Bioinformatics and Expression Analysis (BEA) at Karolinska Institute.

Gene expression profiling

Microarrays were performed at two time-points where pre- and postoperative biopsies were evenly distributed between both time-points. Gene expression profiling was performed using Affymetrix ®Human Gene 2.1 ST oligonucleotide microarrays, as previously described[8]. To identify pathways containing the dysregulated genes, data generated from Affymetrix ® arrays were subjected to enrichment testing using Hallmark gene set enrichment analysis Molecular Signatures Database[11, 12]. The method examines whether differential expression of genetic pathways is higher than expected by chance. It identifies pathways that are affected (up or down regulated) by RT and AFT and provides biological understanding of the processes in the microenvironment.

Analysis I - Gene expression alterations in fat after irradiation:

In order to extract genes with expression patterns profoundly affected by radiotherapy, the 3000 most up or down regulated genes of irradiated tissues compared to non-irradiated tissues were selected, representing the 10 % most dysregulated genes. The 10% threshold for selection was set in order to extract relevant genes only, but still with a sufficient number to be included for further genetic pathway analysis. A fold change (FC) was calculated for each of the 3000 transcripts by comparing gene expression levels of the irradiated with the non-irradiated breast for each patient (FC pre) (Figure 1). Enriched pathways related to irradiation were then identified among the retrieved 3000 genes by using Hallmark analysis, comparing irradiated and non-irradiated preoperative biopsies with paired analysis for each patient.

Analysis II - Gene expression reversal in irradiated fat after AFT:

FC was calculated for the same 3000 transcripts as in Analysis I by comparing the postoperative biopsies of the irradiated and AFT treated breast with the non-irradiated control (FC post). Retrieved gene expression FCs could then be compared before and after AFT (FC pre vs. FC post) with paired t-test for each patient and transcript in order to detect variations caused by AFT with significance level set on 5 % (Figure 1). Genes that were significantly up or down regulated by irradiation preoperatively and then significantly reversed by AFT (FC close to 1) postoperatively were further analysed with Hallmark analysis to identify enriched pathways related to an effect caused by AFT.

Immunohistochemistry

The tissues were routinely fixed in 10% buffered formalin, processed, and embedded in paraffin. Whole tissue sections were used for immunohistochemistry in all samples. Blocks were sectioned at a thickness of 3 µm. CD68 immunostaining[13-15] (PG-M1 clone, Dako, Carpenteria, California) was performed by using a standard immunohistochemistry protocol. The antibody labels human monocytes and macrophages. Immunohistochemistry was carried out using an autostainer (Benchmark XT System, Ventana Medical Systems) per the manufacturer's instructions. All glasses were then scanned and two independent, blinded collaborators used NDP.view2 for manual quantification of stained cells. The density (cells per mm^2) ratio irradiated/non-irradiated was compared pre and post AFT.

Statistics

Students T-test was used to test differences between radiated and non-radiated biopsies (FC pre). Paired analysis with t-test was then performed to compare FC pre and FC post. Qlucore Omics Explorer (www.qlucore.com) and Microsoft excel was used to calculate students T-test. No power calculation was performed since there are now previous data or measured effects of AFT to be based on or compare with.

Results

Patient selection

A total of 30 patients were enrolled and 21 patients completed follow-up according to protocol. Three patients were excluded from further analysis (lack of biopsy pair or death), two patients refused follow-up biopsies and in four patients the preoperative biopsies were felt to be of insufficient quality and these patients were excluded from the protocol. RNA was purified in all collected biopsies (n=108). The biopsy pairs with better RNA quality were retained. Nine patients did not meet the requirements of RNA quality. Two samples were excluded due to insufficient quantity. Ten patients remained in the final analysis with both RNA quality and quantity that met the requirements for microarray analysis of the entire genome (Figure 1, Supplements).

Patient demographics

Median age at the time of AFT treatment was 53.7 years (range 31–70 years) among patients left for microarray analysis. The median radiation dose was at 50 Gy (range 46-60, one patient's dose unknown). Median BMI at baseline was 24.2 (range 20.8-29.1) and had decreased to 22.9 at follow-up biopsy. One patient was a smoker and one patient was hypothyroid. Nine women had hormonal treatment for breast cancer. Median number of AFT treatments was 2.5 (range 1-4) with a median of total fat volume of 160 ml (range 82-322). Median time from last radiotherapy session to AFT was 163 weeks (range 56-740). Median time from AFT to follow-up biopsy was 62 weeks (range 45-119). There were no surgical complications (Table 1).

Overview of expression data

Due to two time-points for microarray analysis, array data were subjected to a "batch-control" analysis showing a negligible effect of the temporal aspect, which further validated the reproducibility of the experiment. The investigated tissues from the non-irradiated control breasts were unchanged over time (from pre to post AFT), but divergent from the irradiated breast both before and after AFT.

Analysis I - Gene expression alterations in fat after irradiation:
The analysis represents gene expression alterations caused by radiotherapy at a median time of 162.6 weeks following radiation exposure. Among these genes 45 pathways were significantly altered (p-value range 1.3×10^{-24} - 0.023) in the Hallmark analysis. The false discovery rate (FDR) q-values were low for all pathways (range 6.4×10^{-23}-0.026). This higher gene set enrichment score was demonstrated for overlapping gene sets designated inflammation, fibrosis, cell adhesion and hypoxia. Epithelial mesenchymal transition (EMT) was the most dysregulated pathway followed by the interferon gamma (IFNγ) response (Figure 2). Connective tissue growth factor (CTGF) was one of the most dysregulated genes ($p<0.001$) and furthermore present in the EMT pathway. The 100 most dysregulated genes are given in supplement Table 1.

Analysis II - Gene expression reversal in irradiated fat after AFT:
The analysis showed that 575 (19 %) of the 3000 genes analysed in Analysis I, had significantly less variation between the respective sides post AFT compared to pre AFT (FC pre vs. FC post). AFT significantly altered FC values for CTGF ($p=0.004$). Hallmark analysis of the 575 genes showed 13 enriched pathways (all previously among the enriched gene sets in Analysis I) (p-value range 1.0×10^{-4}-0.013; FDR range 5.1×10^{-4} - 0.05). Interferon gamma (IFNγ) was most affected by AFT ($p=1.1\times10^{-5}$) followed by hypoxia ($p=6.4\times10^{-5}$) (Figure 2, complete list in supplements Table 2). Genes involved in the IFNγ and hypoxia pathways are displayed in Figure 3.

Immunohistochemistry
Out of the ten patients, one lacked preoperative biopsy for immunohistochemistry and four had not satisfying tissue or stain quality for cell counting in one of four biopsies. Biopsies of the remaining five patients were examined by two blinded evaluators. In all patients except one, the macrophage ratio (irradiated/non-irradiated pre vs. irradiated/non-irradiated post) was lowered after AFT (Figure 4).

Discussion

The current study shows that AFT can alter radiotherapy-induced gene expression patterns in humans. By using a global gene expression strategy, with enrichment testing, it was demonstrated that this effect was most prominent in pathways involved in inflammation, fibrosis and hypoxia. The prospective setting with validated internal controls of the patient´s non-irradiated breast provides a good opportunity to unravel the underlying mechanisms associated with AFT in irradiated humans. The study also includes a global analysis of genes that are dysregulated by irradiation in human adipose tissue, which to our knowledge has not been described previously. However, the findings are equally supported by earlier studies in other tissues and/or species. The initial finding of the most enriched pathways in the irradiated breast before AFT was EMT, previously known to be activated by irradiation in other tissues[7, 16-18]. EMT has also been shown to be activated by CTGF[19], which was furthermore one of the most highly expressed genes after irradiation in the current study. In a recent review by Kumar *et al* about AFT in radiation-induced fibrosis[17], one-third of studies recognized CTGF to be highly involved in fibrotic cascades, and, three studies suggested hypoxia as the stimulus for the fibrotic response. However, none of these studies included in this review were in human tissues. Other recent work by Khan *et al* has shown that reducing CTGF gene expression using a lentivirally-delivered, small hairpin RNA, *in vivo*, can protect flap tissues from fibrosis post-radiotherapy, which further supports a pivotal role for CTGF in this context. [20]. Therapeutic studies using a monoclonal antibody targeting CTGF have also shown reductions in the fibrotic burden in RT-induced pulmonary fibrosis[21]. Our own findings, in this study, of highly dysregulated CTGF gene expression in human breast adipose tissue is therefore a highly relevant contribution to the literature.

The second most enriched pathway in the analysis of AFT treatment effects (Analysis II) was hypoxia, where a majority of the involved, dysregulated genes reached FC values closer to 1 after AFT (1.0 = no difference between non-irradiated and previously irradiated breasts). This is particularly interesting since hypoxia[22] is regarded as a hallmark of radiation induced fibrosis[23] and caused by a post-radiation vasculopathy as shown by others, and, us[8, 24]. Tissue hypoxia is associated with perivascular matrix deposition and the induction of extracellular matrix synthesis[17, 25]. Luan *et al* and Garza *et al* showed less hypovascularity in previously irradiated skin after AFT in mice[6, 26]. In addition, Garza *et al* also showed decreased dermal thickening and collagen deposition. Rigotti *et al* treated 20 patients with severe-to-irreversible adverse effects of irradiation (LENT-SOMA 3-4) with autologous purified fat cell transplantation with all but one patient showing a significant improvement in symptoms. Electron microscopy prior to AFT showed patterns consistent with radiation-induced ischaemic lesions and scleroderma and, post-operatively, they observed a gradual improvement and normalisation of the microcirculation with greater hydration and less fibrosis. They also observed increased macrophage influx post-RT and reported a reduction in this post-AFT. These reports support the validity of our findings that AFT reduced hypoxia and fibrosis pathway expression, together with reduced macrophage density, and contributes to a biological explanation for the clinical success of AFT.

Interestingly, IFNγ response, known to be associated with inflammatory responses to ionizing radiation[22, 27, 28], was the second most enriched pathway in analysis of radiation effects (Analysis I) and the most enriched pathway in analysis of AFT treatment effects (Analysis II). Inflammatory cytokines play a pivotal role in the development of fibrosis. The current study clearly shows that both innate and adaptive inflammatory networks are activated years after radiotherapy in adipose tissue. This is in itself a valuable finding since there is a paucity of studies on the effects of radiotherapy in human adipose tissue. Poglio *et al* could for instance show a severe decrease in proliferating cells, as well as a significant increase in apoptotic cells in inguinal fat pads, following radiation exposure in mice. Decrease in the proliferation and differentiation capacities of non-hematopoietic progenitors was also observed following irradiation. The study indicates that subcutaneous adipose tissue is very sensitive to irradiation, leading to a profound alteration of its developmental potential. The authors suggest that AFT with mesenchymal stem cells may have a role to reverse the injury as a potential treatment for RIF in skin and subcutaneous fat[29]. A trend towards reduced macrophage density was observed in irradiated adipose tissue after AFT in the current study. We encourage further in depth studies looking at the specific role of macrophages in adipose tissue after irradiation and AFT since macrophages are known to be master regulators of inflammation and fibrosis[30].

Limitations of the study need to be acknowledged. The global gene expression strategy describes several pathways involved in radiation injury as well as the effect of AFT on irradiated tissues, but does not include in depth analysis of a specific mechanism. However, the study was intentionally designed to unravel the main networks activated in a wider perspective. The number of patients may be regarded as a limitation, but the internal control of the non-irradiated breast both before and after AFT enables paired analysis and increases the power significantly. This is in line with previous studies performed on conduit vessels with similar design and sample size[8, 24].

Taken together, it has been studied how radiotherapy can cause alterations in global gene expression patterns by comparing irradiated and non-irradiated adipose tissue harvested from the same patient at the same time, and thereby eliminating inter-individual factors. Long-term alterations in the described gene expression patterns following AFT to the irradiated breast have furthermore been studied, still with a simultaneous control biopsy from the contralateral non-irradiated breast. Our results clearly show enriched gene expression pathways associated with sustained inflammation, fibrosis and hypoxia several years after radiation exposure in the irradiated breast compared to the control. It has further been shown that AFT attenuated radiation-induced dysregulated gene expression patterns related to the same pathways. We believe that effect caused by AFT on inflammation and hypoxia, together with the identification of a consistent and rather uniform pattern of differentially expressed genes between radiated and non-radiated breasts, have generated new perspectives for future research. The study has also generated biological support in humans for the potential of AFT to be used to ameliorate RIF. We therefore welcome further studies regarding biological effects and clinical outcome following AFT.

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No preregistration exists for the reported studies reported in this article.

Tables and Figures

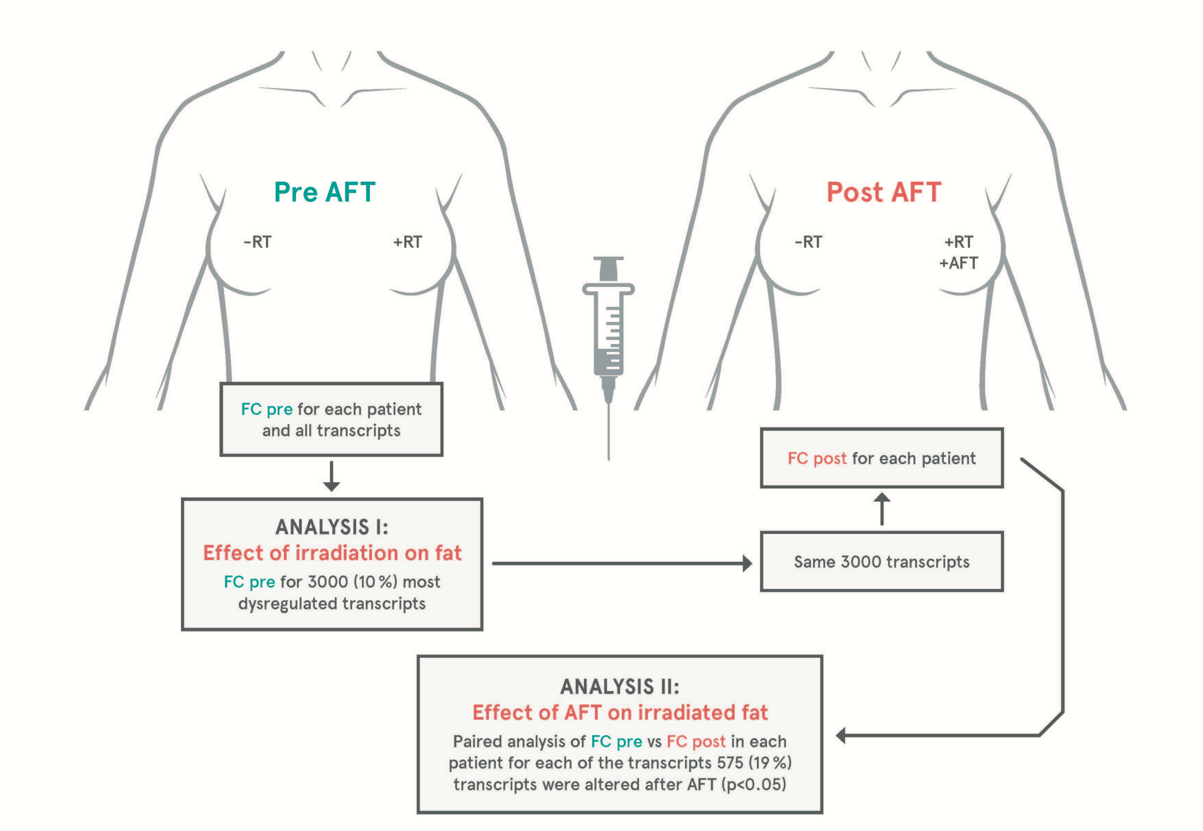


Figure 1. A global gene expression strategy was conducted to unravel altered pathways. Analysis I (RT vs. no RT): Initial selection of the 3000 most differentially expressed genes in irradiated adipose tissue compared to internal controls. Analysis II (No RT vs. RT vs. RT + AFT): The effect of AFT was determined by comparison of fold change values before and after AFT (Fold change=1 represents no difference between the irradiated and non irradiated breast). FC=Fold change, -RT=non-irradiated (control), +RT=irradiated, +RT+AFT=irradiated and AFT treated

Previous breast surgery (n)	10	
*Mastectomy**	4	
Mastectomy + delayed reconstruction with expander prosthesis	1	
BCS	5	
Time from irradiation to preop biopsy/first AFT (mos)	37.4	12.9-170.2
Radiation dose (Gy)**	50	46-60
Age at preop biopsy and first AFT (y)	53.7	31.3-70
Fat injected (ml)	160.0	82-322
Number of treatments (n)	2.5	1-4
BMI first biopsy (kg/m2)	24.2	20.8-29.1
BMI follow-up biopsy (kg/m2)	22.9	20.5-28.8
Time from AFT to postop biopsy (mos)	14.2	10.3-27.3

Table 1. Patient characteristics (n=10) Median and range are given for continuous values. AFT=autologous fat transplantation, BMI=body mass index, RT=radiotherapy *The patients underwent delayed breast reconstruction with expander prosthesis between pre- and postoperative biopsies **One patients radiation dose is not known

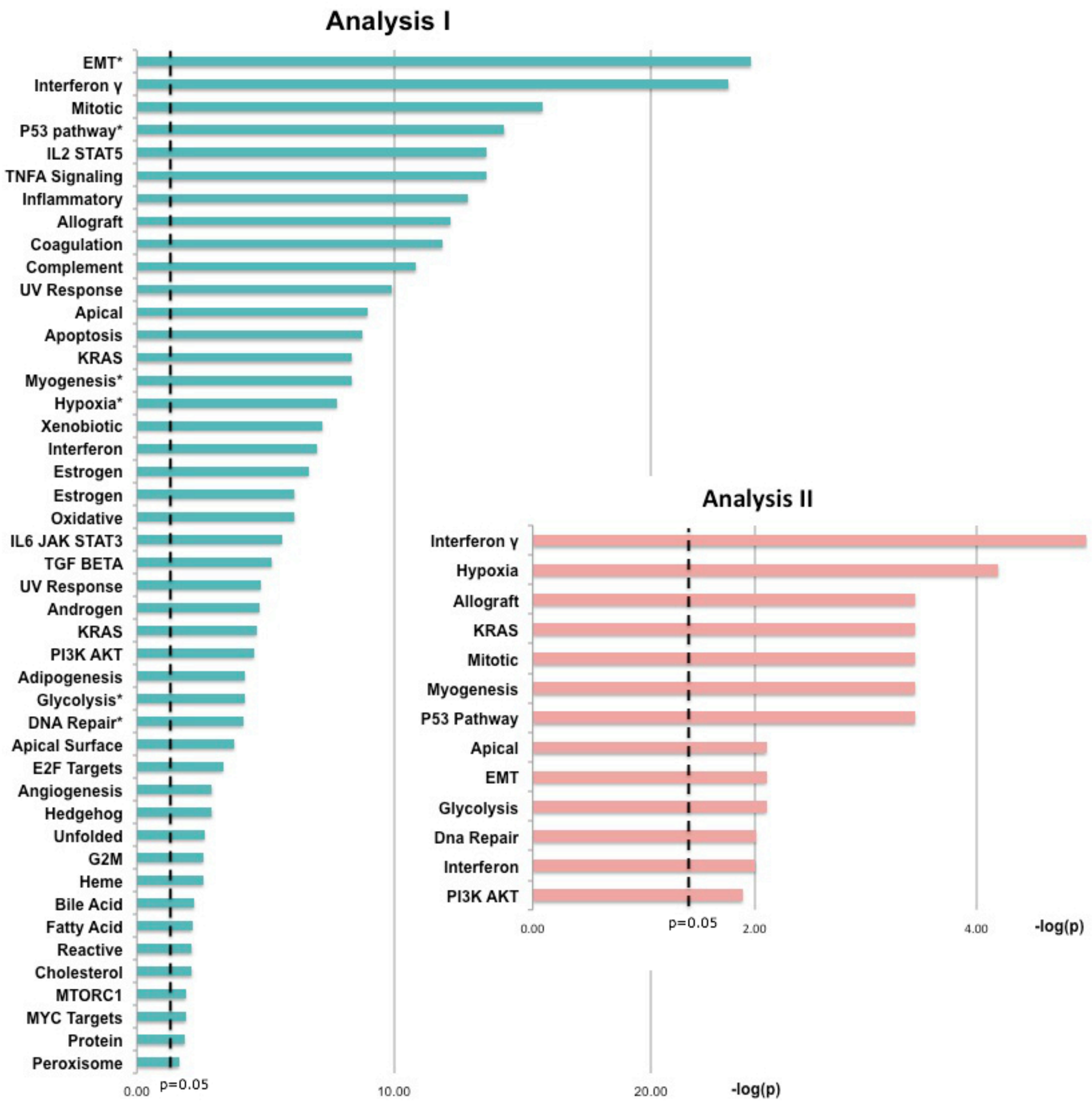


Figure 2. Significantly enriched pathways after Hallmark analysis, sorted by increasing p-values (-log(p)), showing the most significantly altered gene expression patterns for Analysis I (n=45) and II (n=13). The blue bars (Analyse I) shows –log(p) for difference in gene expression between preoperative (irradiated vs. non-irradiated) biopsies. The red bars shows –log(p) for difference between fold change fore preoperative and postoperative biopsies. The dotted vertical line shows the 0.05 significance level.*Differentially expressed pathways significantly affected by both irradiation and AFT.

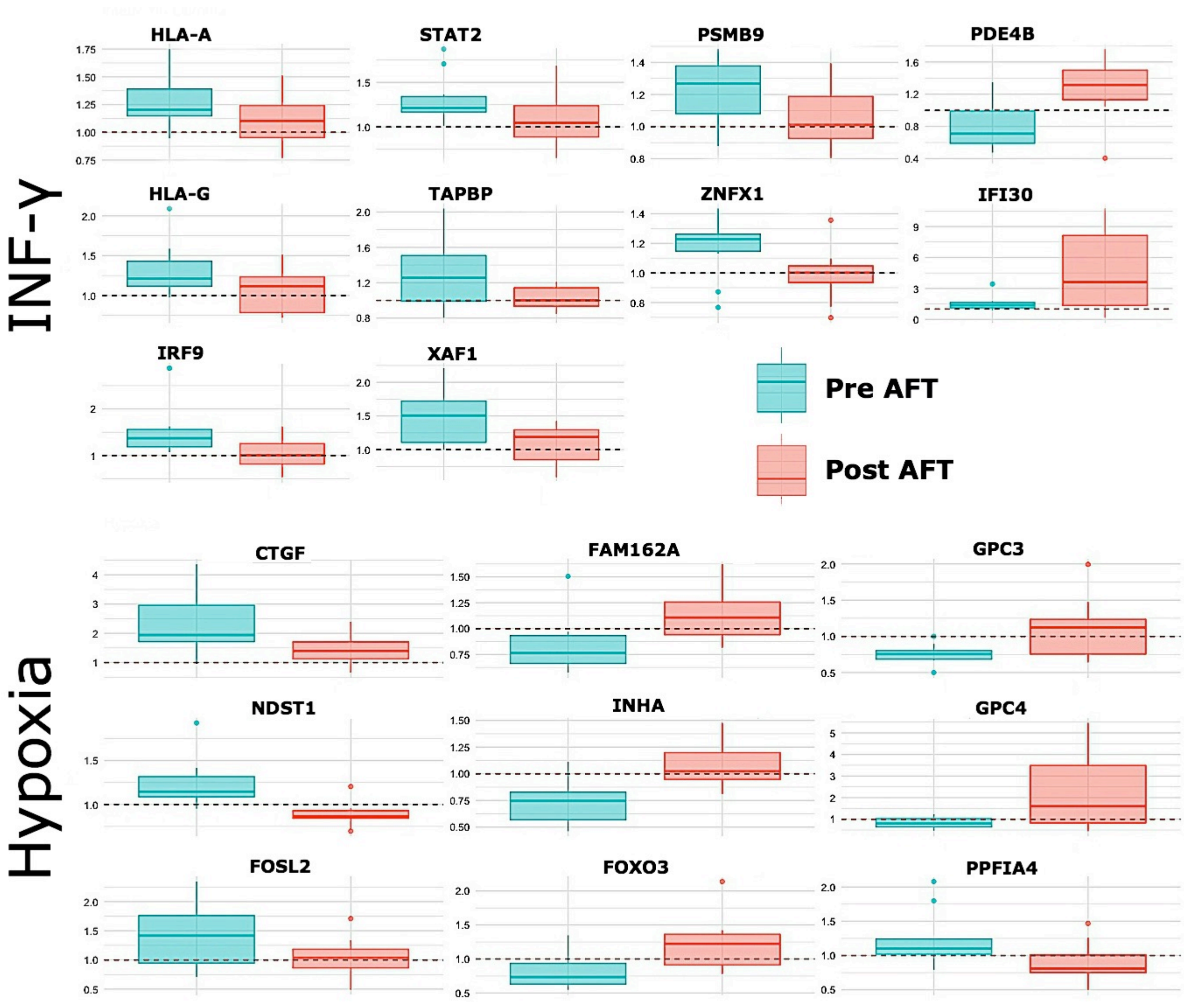
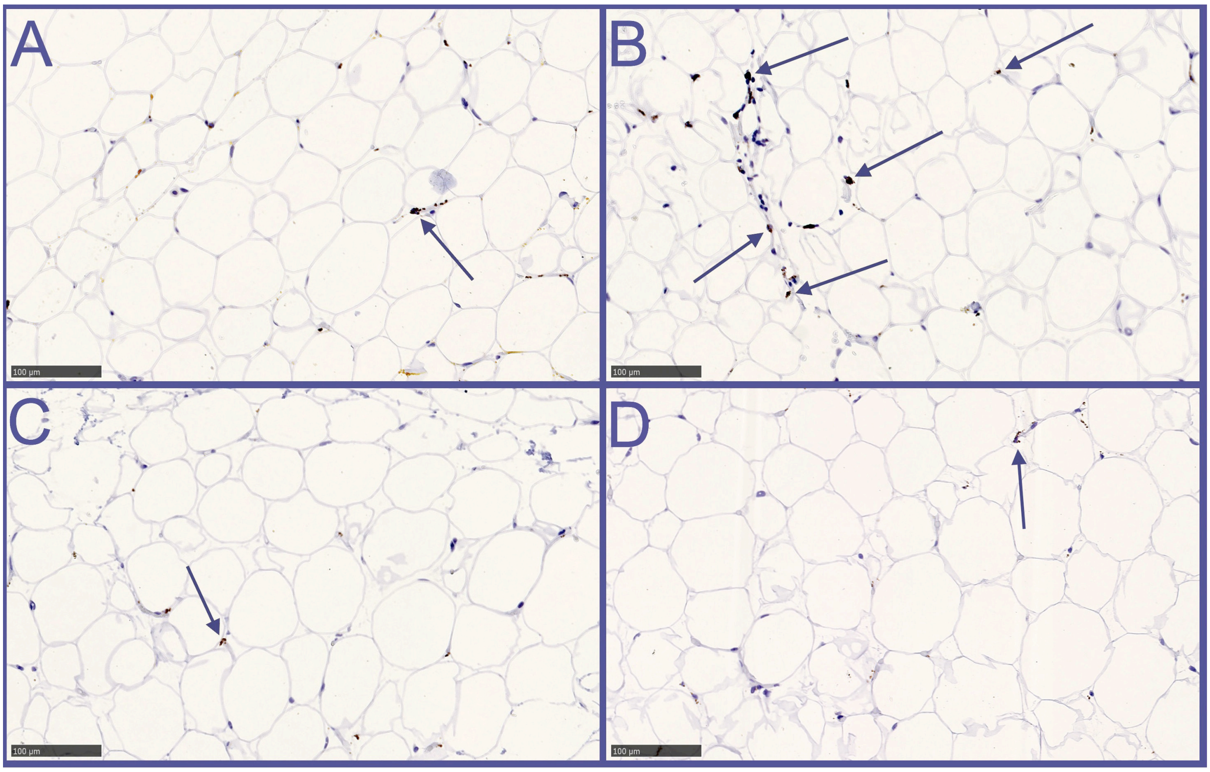


Figure 3: Effect of AFT on mRNA expression patterns for individual genes involved in interferon gamma response and hypoxia pathways. The Y-axes shows fold change (FC). FC 1.0 indicates no difference in gene expression level between the compared biopsy pair (irradiated breast compared to the non-irradiated control). Deviation from FC 1.0 means that the gene is dysregulated (up or down regulated) compared to the non-radiated control breast. Blue box: comparison of gene expression before AFT. Pink box: comparison of gene expression after AFT.



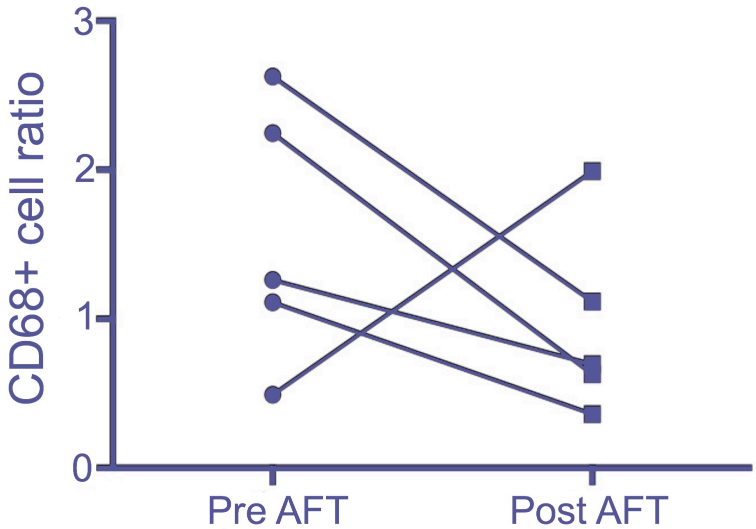


Figure 4. Immunohistochemistry for CD68 staining. A) Non-irradiated, pre AFT B) Irradiated, pre AFT C) Non-irradiated, post AFT D) Irradiated, post AFT Arrows indicate macrophages. (scale = 250 and 50 µm). Graph: Cell density (cells/mm2) ratio of CD68+ cells in irradiated and non-irradiated tissues, before and after AFT. Four out of five patient had a lowered ratio after AFT treatment.

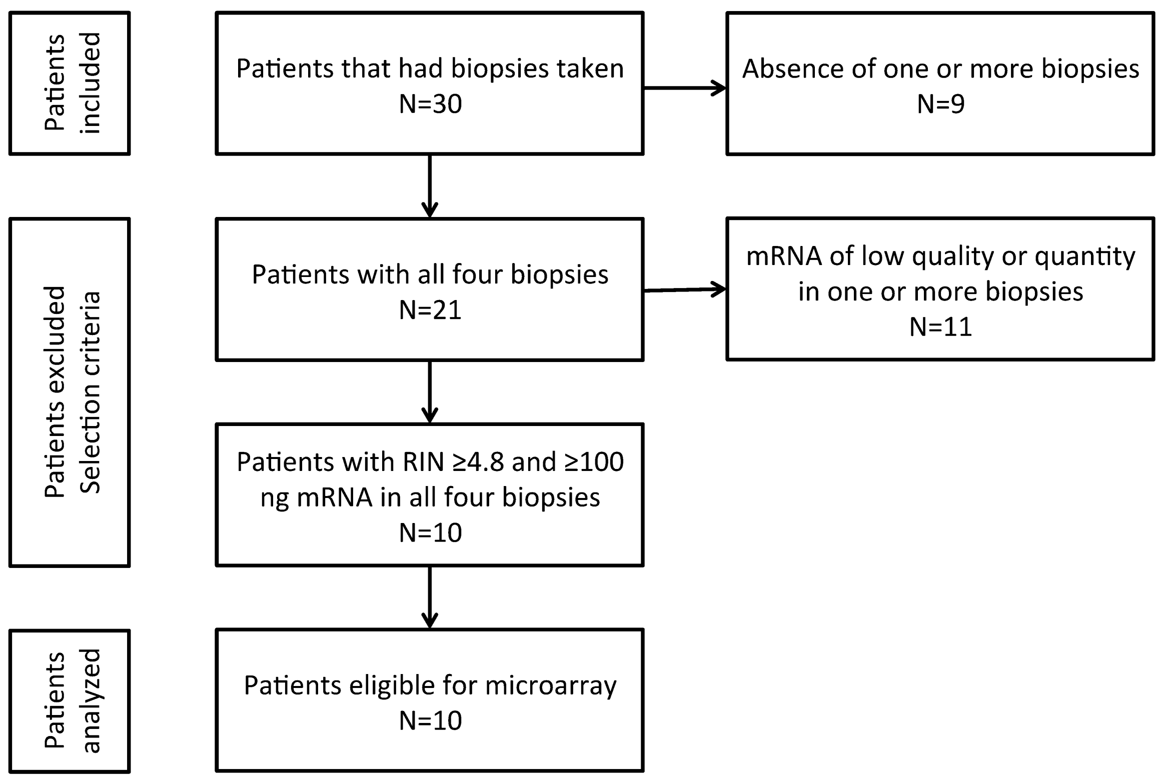
References

1. Sinno S, Wilson S, Brownstone N, Levine SM. Current Thoughts on Fat Grafting: Using the Evidence to Determine Fact or Fiction. *Plastic and Reconstructive Surgery* 2016;**137**(3): 818-824.
2. Costantino A, Fioramonti P, Ciotti M, Onesti MG. Lipofilling in skin affected by radiodermatitis: clinical and ultrasound aspects. Case report. *Il Giornale di chirurgia* 2012;**33**(5): 186-190.
3. Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M, Pasini A, Sbarbati A. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plastic and Reconstructive Surgery* 2007;**119**(5): 1409-1422; discussion 1423-1404.
4. Mojallal A, Lequeux C, Shipkov C, Breton P, Foyatier JL, Braye F, Damour O. Improvement of skin quality after fat grafting: clinical observation and an animal study. *Plastic and Reconstructive Surgery* 2009;**124**(3): 765-774.
5. Forcheron F, Agay D, Scherthan H, Riccobono D, Herodin F, Meineke V, Drouet M. Autologous adipocyte derived stem cells favour healing in a minipig model of cutaneous radiation syndrome. *PloS one* 2012;**7**(2): e31694.
6. Garza RM, Paik KJ, Chung MT, Duscher D, Gurtner GC, Longaker MT, Wan DC. Studies in fat grafting: Part III. Fat grafting irradiated tissue--improved skin quality and decreased fat graft retention. *Plastic and Reconstructive Surgery* 2014;**134**(2): 249-257.
7. Weigel C, Schmezer P, Plass C, Popanda O. Epigenetics in radiation-induced fibrosis. *Oncogene* 2015;**34**(17): 2145-2155.
8. Halle M, Gabrielsen A, Paulsson-Berne G, Gahm C, Agardh HE, Farnebo F, Tornvall P. Sustained inflammation due to nuclear factor-kappa B activation in irradiated human arteries. *Journal of the American College of Cardiology* 2010;**55**(12): 1227-1236.
9. Tall J, Bjorklund TC, Skogh AC, Arnander C, Halle M. Vascular Complications After Radiotherapy in Head and Neck Free Flap Reconstruction: Clinical Outcome Related to Vascular Biology. *Annals of plastic surgery* 2015;**75**(3): 309-315.
10. Schultz I, Lindegren A, Wickman M. Improved shape and consistency after lipofilling of the breast: patients' evaluation of the outcome. *Journal of plastic surgery and hand surgery* 2012;**46**(2): 85-90.
11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* 2005;**102**(43): 15545-15550.
12. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdottir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics (Oxford, England)* 2011;**27**(12): 1739-1740.
13. Falini B FL, Pileri S, Stein H, Dürkop H, Pasqualucci L, et al. M15.1. PG-M1 a new mAb directed against a fixative-resistant, macrophage-restricted epitope of the CD68 molecule. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 928-30.
14. Falini B, Flenghi L, Pileri S, Gambacorta M, Bigerna B, Durkop H, Eitelbach F, Thiele J, Pacini R, Cavaliere A, et al. PG-M1: a new monoclonal antibody directed against a fixative-resistant epitope on the macrophage-restricted form of the CD68 molecule. *The American journal of pathology* 1993;**142**(5): 1359-1372.

15. Cordell JL FB, Flenghi L, Jones DB, Pileri S, Radzun HJ, et al. M15. CD68 cluster workshop report. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 925-7.
16. Straub JM, New J, Hamilton CD, Lominska C, Shnayder Y, Thomas SM. Radiation-induced fibrosis: mechanisms and implications for therapy. *Journal of cancer research and clinical oncology* 2015;**141**(11): 1985-1994.
17. Kumar R, Griffin M, Adigbli G, Kalavrezos N, Butler PE. Lipotransfer for radiation-induced skin fibrosis. *The British journal of surgery* 2016;**103**(8): 950-961.
18. Kawamoto A, Yokoe T, Tanaka K, Saigusa S, Toiyama Y, Yasuda H, Inoue Y, Miki C, Kusunoki M. Radiation induces epithelial-mesenchymal transition in colorectal cancer cells. *Oncology reports* 2012;**27**(1): 51-57.
19. Sonnylal S, Xu S, Jones H, Tam A, Sreeram VR, Ponticos M, Norman J, Agrawal P, Abraham D, de Crombrugghe B. Connective tissue growth factor causes EMT-like cell fate changes in vivo and in vitro. *Journal of cell science* 2013;**126**(Pt 10): 2164-2175.
20. Khan AA, Paget JT, McLaughlin M, Kyula JN, Wilkinson MJ, Pencavel T, Mansfield D, Roulstone V, Seth R, Halle M, Somaiah N, Boult JKR, Robinson SP, Pandha HS, Vile RG, Melcher AA, Harris PA, Harrington KJ. Genetically modified lentiviruses that preserve microvascular function protect against late radiation damage in normal tissues. *Science translational medicine* 2018;**10**(425).
21. Bickelhaupt S, Erbel C, Timke C, Wirkner U, Dadrich M, Flechsig P, Tietz A, Pfohler J, Gross W, Peschke P, Hoeltgen L, Katus HA, Grone HJ, Nicolay NH, Saffrich R, Debus J, Sternlicht MD, Seeley TW, Lipson KE, Huber PE. Effects of CTGF Blockade on Attenuation and Reversal of Radiation-Induced Pulmonary Fibrosis. *Journal of the National Cancer Institute* 2017;**109**(8).
22. Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. *Radiation research* 2012;**178**(6): 505-523.
23. Choi SH, Hong ZY, Nam JK, Lee HJ, Jang J, Yoo RJ, Lee YJ, Lee CY, Kim KH, Park S, Ji YH, Lee YS, Cho J, Lee YJ. A Hypoxia-Induced Vascular Endothelial-to-Mesenchymal Transition in Development of Radiation-Induced Pulmonary Fibrosis. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2015;**21**(16): 3716-3726.
24. Christersdottir Bjorklund T, Reilly SJ, Gahm C, Bottazzi B, Mantovani A, Tornvall P, Halle M. Increased long-term expression of pentraxin 3 in irradiated human arteries and veins compared to internal controls from free tissue transfers. *Journal of translational medicine* 2013;**11**: 223.
25. Hoving S, Heeneman S, Gijbels MJ, Te Poele JA, Visser N, Cleutjens J, Russell NS, Daemen MJ, Stewart FA. Irradiation induces different inflammatory and thrombotic responses in carotid arteries of wildtype C57BL/6J and atherosclerosis-prone ApoE(-/-) mice. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 2012;**105**(3): 365-370.
26. Luan A, Duscher D, Whittam AJ, Paik KJ, Zielins ER, Brett EA, Atashroo DA, Hu MS, Lee GK, Gurtner GC, Longaker MT, Wan DC. Cell-Assisted Lipotransfer Improves Volume Retention in Irradiated Recipient Sites and Rescues Radiation-Induced Skin Changes. *Stem cells (Dayton, Ohio)* 2016;**34**(3): 668-673.
27. Di Maggio FM, Minafra L, Forte GI, Cammarata FP, Lio D, Messa C, Gilardi MC, Bravata V. Portrait of inflammatory response to ionizing radiation treatment. *Journal of inflammation (London, England)* 2015;**12**: 14.

28. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nature reviews Cancer* 2015;**15**(7): 409-425.
29. Poglio S, Galvani S, Bour S, Andre M, Prunet-Marcassus B, Penicaud L, Casteilla L, Cousin B. Adipose tissue sensitivity to radiation exposure. *The American journal of pathology* 2009;**174**(1): 44-53.
30. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Seminars in liver disease* 2010;**30**(3): 245-257.

Supplements



Supplement Figure 1. Flow diagram. RIN=RNA integrity number

Single Gene Symbol	Gene Title	FC	p-value
GPR126	G protein-coupled receptor 126	1.360	2.88E-06
LMO3	LIM domain only 3 (rhombotin-like 2)	0.593	2.10E-05
OSBPL3	oxysterol binding protein-like 3	1.487	2.24E-05
ASAP1	ArfGAP with SH3 domain. ankyrin repeat and PH domain 1	1.385	2.79E-05
KCNK17	potassium channel. subfamily K. member 17	1.418	4.68E-05
PTPRB	protein tyrosine phosphatase. receptor type. B	1.560	5.36E-05
BMP6	bone morphogenetic protein 6	1.561	7.79E-05
BACH1-IT3	BACH1 intronic transcript 3 (non-protein coding)	1.551	9.90E-05
PIK3R5	phosphoinositide-3-kinase. regulatory subunit 5	1.304	1.13E-04
ZMAT3	zinc finger. matrin-type 3	1.909	1.22E-04
SPATA18	spermatogenesis associated 18	2.100	1.95E-04
MNS1	meiosis-specific nuclear structural 1	0.776	1.96E-04
TMEM105	transmembrane protein 105	0.798	2.18E-04
DDB2	damage-specific DNA binding protein 2. 48kDa	1.800	2.25E-04
NWD1	NACHT and WD repeat domain containing 1	0.760	2.31E-04
SHROOM4	shroom family member 4	1.319	2.67E-04
ANG	angiogenin. ribonuclease. RNase A family. 5 /// 4	0.760	2.71E-04
CYP4B1	cytochrome P450. family 4. subfamily B. polypeptide 1	0.560	2.95E-04
LOC101060431	E3 SUMO-protein ligase PIAS3-like /// protein inhibitor of activated STAT	1.443	3.04E-04
FILIP1L	filamin A interacting protein 1-like	1.458	3.15E-04
C22orf24	chromosome 22 open reading frame 24	1.219	3.21E-04
WWC3	WWC family member 3	1.275	3.23E-04
DHX38	DEAH (Asp-Glu-Ala-His) box polypeptide 38	1.248	3.24E-04
WNT2B	wingless-type MMTV integration site family. member 2B	1.386	3.51E-04
SEC24D	SEC24 family. member D (S. cerevisiae)	1.390	3.62E-04
ELL	elongation factor RNA polymerase II	1.535	3.75E-04
CBX2	chromobox homolog 2	0.811	3.77E-04
TRIM22	tripartite motif containing 22	1.499	3.95E-04

AC018730.1	uncharacterized LOC100506421	0.800	3.98E-04
TBL1X	transducin (beta)-like 1X-linked	0.888	4.22E-04
RPL27	ribosomal protein L27	0.811	4.29E-04
PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	1.230	4.40E-04
CENPP	centromere protein P /// inositol 1.3.4.5.6-pentakisphosphate 2-kinase	1.304	4.56E-04
MYO1E	myosin IE	1.308	4.67E-04
ELK3	ELK3. ETS-domain protein (SRF accessory protein 2)	1.328	4.78E-04
CXorf36	chromosome X open reading frame 36	1.502	4.94E-04
PTCHD4	patched domain containing 4	2.162	5.36E-04
HSPA7	heat shock 70kDa protein 7 (HSP70B)	1.871	5.71E-04
DEFB135	defensin. beta 135	0.763	6.03E-04
HYMAI	hydatidiform mole associated and imprinted (non-protein coding)	1.292	6.64E-04
FUCA2	fucosidase. alpha-L- 2. plasma	1.377	6.68E-04
PTPN13	protein tyrosine phosphatase. non-receptor type 13	0.818	7.19E-04
FIP1L1	FIP1 like 1 (S. cerevisiae) /// platelet-derived growth factor receptor	0.548	7.21E-04
IRF9	interferon regulatory factor 9	1.486	7.42E-04
MT4	metallothionein 4	1.483	7.60E-04
MIR591	microRNA 591	1.432	7.84E-04
HERC2P9	hect domain and RLD 2 pseudogene 9	1.545	8.06E-04
SLC13A3	solute carrier family 13. member 3	1.397	8.10E-04
F2R	coagulation factor II (thrombin) receptor	2.320	8.56E-04
AGTR1	angiotensin II receptor. type 1	0.687	8.70E-04
SAP18	Sin3A-associated protein. 18kDa	0.895	8.82E-04
PAIP2B	poly(A) binding protein interacting protein 2B	0.719	9.04E-04
EDA2R	ectodysplasin A2 receptor	2.716	9.37E-04
ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)	1.431	9.43E-04
ZNF697	zinc finger protein 697	0.721	9.49E-04
PBX4	pre-B-cell leukemia homeobox 4	0.774	9.61E-04
STAT2	signal transducer and activator of transcription 2. 113kDa	1.308	9.66E-04
CTGF	connective tissue growth factor	2.320	9.68E-04

LOC440461	Rho GTPase activating protein 27 pseudogene	1.401	9.78E-04
MTMR9LP	myotubularin related protein 9-like. pseudogene	1.488	9.91E-04
MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	1.370	1.03E-03
SLC8A1	solute carrier family 8 (sodium/calcium exchanger). member 1	1.262	1.11E-03
MDM2	MDM2 oncogene. E3 ubiquitin protein ligase	1.715	1.13E-03
RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5	1.397	1.15E-03
GPC3	glypican 3	0.754	1.15E-03
NSUN2	NOP2/Sun RNA methyltransferase family. member 2	1.270	1.20E-03
SPTAN1	spectrin. alpha. non-erythrocytic 1	1.185	1.20E-03
CBS	cystathionine-beta-synthase	0.824	1.23E-03
LOC283177	uncharacterized LOC283177	1.320	1.23E-03
RRAGD	Ras-related GTP binding D	0.749	1.23E-03
TRIM66	tripartite motif containing 66	1.276	1.24E-03
SYNE1	spectrin repeat containing. nuclear envelope 1	0.848	1.30E-03
OTTHUMG00000162873	NULL	0.785	1.31E-03
MCOLN3	mucolipin 3	0.531	1.31E-03
OMG	oligodendrocyte myelin glycoprotein	1.839	1.32E-03
RPL7	ribosomal protein L7	1.367	1.33E-03
RPS27	ribosomal protein S27	0.720	1.34E-03
MIR3689F	microRNA 3689f	1.464	1.35E-03
PXMP2	peroxisomal membrane protein 2. 22kDa	0.764	1.37E-03
XAF1	XIAP associated factor 1	1.501	1.44E-03
HERC2P4	hect domain and RLD 2 pseudogene 4	1.448	1.44E-03
TM4SF1	transmembrane 4 L six family member 1	1.403	1.46E-03
LOC730811	uncharacterized LOC730811	1.201	1.46E-03
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A. ionotropic	1.374	1.48E-03
ABCC5	ATP-binding cassette. sub-family C (CFTR/MRP). member 5	1.303	1.51E-03
CCL2	chemokine (C-C motif) ligand 2	1.788	1.52E-03
BCL6B	B-cell CLL/lymphoma 6. member B	1.691	1.53E-03
IL18BP	interleukin 18 binding protein	1.496	1.59E-03

NDP-AS1	NDP antisense RNA 1	1.313	1.62E-03
LSAMP	limbic system-associated membrane protein	1.231	1.62E-03
ADCY4	adenylate cyclase 4	1.463	1.65E-03
SLC12A9	solute carrier family 12 (potassium/chloride transporters). member 9	0.821	1.65E-03
EXD2	exonuclease 3'-5' domain containing 2	0.796	1.67E-03
RNU7-72P	RNA. U7 small nuclear 72 pseudogene	1.410	1.67E-03
EID3	EP300 interacting inhibitor of differentiation 3	1.232	1.69E-03
CCDC73	coiled-coil domain containing 73	1.170	1.69E-03
FAM83A-AS1	FAM83A antisense RNA 1	1.367	1.70E-03
STX2	syntaxin 2	1.265	1.70E-03
HERC2P2	hect domain and RLD 2 pseudogene 2	1.476	1.70E-03
RPS27L	ribosomal protein S27-like	1.311	1.71E-03

Supplement Table 1. One-hundred most dysregulated genes in Analysis I. Irradiated compared to non-irradiated biopsies. FC=Fold change

Analysis I

Gene Set Name	p-value	FDR q-value	Genes in Gene Set	Genes in Overlap
EPITHELIAL MESENCHYMAL TRANSITION*	1,29E-24	6,44E-23	200	52
INTERFERON GAMMA RESPONSE*	9,44E-24	2,36E-22	200	51
MITOTIC SPINDLE*	1,69E-16	2,81E-15	200	42
P53 PATHWAY*	5,01E-15	6,27E-14	200	40
IL2 STAT5 SIGNALING	2,61E-14	2,18E-13	200	39
TNFA SIGNALING VIA NFKB	2,61E-14	2,18E-13	200	39
INFLAMMATORY RESPONSE	1,32E-13	9,40E-13	200	38
ALLOGRAFT REJECTION*	6,43E-13	4,02E-12	200	37
COAGULATION	1,29E-12	7,19E-12	138	30
COMPLEMENT	1,39E-11	6,94E-11	200	35
UV RESPONSE DN	1,18E-10	5,35E-10	144	28
APICAL JUNCTION*	1,08E-09	4,49E-09	200	32
APOPTOSIS	1,70E-09	6,56E-09	161	28
KRAS SIGNALING UP*	4,28E-09	1,43E-08	200	31
MYOGENESIS*	4,28E-09	1,43E-08	200	31
HYPOXIA*	1,64E-08	5,11E-08	200	30
XENOBIOTIC METABOLISM	6,02E-08	1,77E-07	200	29
INTERFERON ALPHA RESPONSE*	9,66E-08	2,68E-07	97	19
ESTROGEN RESPONSE EARLY	2,13E-07	5,61E-07	200	28
ESTROGEN RESPONSE LATE	7,25E-07	1,73E-06	200	27
OXIDATIVE PHOSPHORYLATION	7,25E-07	1,73E-06	200	27
IL6 JAK STAT3 SIGNALING	2,34E-06	5,33E-06	87	16
TGF BETA SIGNALING	5,53E-06	1,20E-05	54	12
UV RESPONSE UP	1,51E-05	3,16E-05	158	21
ANDROGEN RESPONSE	1,71E-05	3,43E-05	101	16
KRAS SIGNALING DN	2,22E-05	4,27E-05	200	24
PI3K AKT MTOR SIGNALING*	2,82E-05	5,21E-05	105	16

Analysis II

Gene Set Name	p-value	FDR q-value	Genes in Gene Set	Genes in Overlap
INTERFERON GAMMA RESPONSE	1,02E-05	5,08E-04	200	10
HYPOXIA	6,38E-05	1,60E-03	200	9
ALLOGRAFT REJECTION	3,59E-04	2,57E-03	200	8
KRAS SIGNALING UP	3,59E-04	2,57E-03	200	8
MITOTIC SPINDLE	3,59E-04	2,57E-03	200	8
MYOGENESIS	3,59E-04	2,57E-03	200	8
P53 PATHWAY	3,59E-04	2,57E-03	200	8
APICAL JUNCTION	7,86E-03	3,93E-02	200	6
EPITHELIAL MESENCHYMAL TRANSITION	7,86E-03	3,93E-02	200	6
GLYCOLYSIS	7,86E-03	3,93E-02	200	6
DNA REPAIR	9,71E-03	4,11E-02	150	5
INTERFERON ALPHA RESPONSE	9,87E-03	4,11E-02	97	4
PI3K AKT MTOR SIGNALING	1,29E-02	4,96E-02	105	4

ADIPOGENESIS	6,35E-05	1,10E-04	200	23
GLYCOLYSIS*	6,35E-05	1,10E-04	200	23
DNA REPAIR*	7,36E-05	1,23E-04	150	19
APICAL SURFACE	1,62E-04	2,62E-04	44	9
E2F TARGETS	4,53E-04	7,08E-04	200	21
ANGIOGENESIS	1,19E-03	1,75E-03	36	7
HEDGEHOG SIGNALING	1,19E-03	1,75E-03	36	7
UNFOLDED PROTEIN RESPONSE	2,33E-03	3,33E-03	113	13
G2M CHECKPOINT	2,65E-03	3,58E-03	200	19
HEME METABOLISM	2,65E-03	3,58E-03	200	19
BILE ACID METABOLISM	5,99E-03	7,88E-03	112	12
FATTY ACID METABOLISM	7,10E-03	8,88E-03	158	15
REACTIVE OXIGEN SPECIES PATHWAY	7,23E-03	8,88E-03	49	7
CHOLESTEROL HOMEOSTASIS	7,28E-03	8,88E-03	74	9
MTORC1 SIGNALING	1,26E-02	1,46E-02	200	17
MYC TARGETS V1	1,26E-02	1,46E-02	200	17
PROTEIN SECRETION	1,39E-02	1,58E-02	96	10
PEROXISOME	2,31E-02	2,57E-02	104	10

Supplement Table 2. Enriched pathways after Hallmark analysis, sorted by increasing p-values, showing the most significantly altered gene expression patterns for Analysis I (n=45) and II (n=13). *Differentially expressed pathways significantly affected by both irradiation and AFT.

Till Dig som genomgått fettransplantation till bröstet

Vi som arbetar med fettransplantationer (lipofilling) på Kliniken för Rekonstruktiv Plastikkirurgi Karolinska Universitetssjukhuset vill gärna utvärdera resultatet av operationerna och vore tacksamma om Du ville ta Dig tid att besvara nedanstående frågor.

Svaren på frågorna kommer att sammanställas av oss plastikkirurger som arbetar med fettransplantation och endast presenteras för utomstående på ett avidentifierat sätt och som sammanslagna data.

Du kan skicka svaret till oss i det bifogade kuvertet, som inte behöver frankeras.

Ditt namn och födelsenummer:

....

Ringa in det svarsalternativ som stämmer bäst nedan.

Till vilket bröst har fett transplanterats? Höger Vänster Båda

Hur många gånger har Du genomgått fettransplantation?

....

Om Du har fettransplanterats fler än en gång – märkte Du någon skillnad mellan gångerna?

Ja Nej Vet inte

Hur?...

Har du tidigare fått strålbehandling mot det bröst som fettransplanterats?

Ja Nej Vet inte

Vad tycker Du om resultatet av fettransplantationerna när det gäller bröstets konsistens? (Har det blivit mjukare, känns det mer naturligt?)

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Vad tycker Du om resultatet av fettransplantationerna när det gäller bröstets storlek?

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Vad tycker Du om resultatet av fettransplantationerna när det gäller bröstets form?

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Vad tycker Du om resultatet av fettransplantationerna när det gäller känsel på bröstets hud?

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Vad tycker Du om resultatet av fettransplantationerna när det gäller bröstets hudkvalitet?

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Vad tycker Du om resultatet av fettransplantationerna när det gäller eventuella ojämnheter i bröstets form?

Mycket bättre Bättre Oförändrat Sämre Mycket sämre

Har Du några kvarvarande smärtor ifrån det ställe varifrån fettet togs?

Ja Nej Vet inte

Kommentar:..
....

Har Du några andra kvarvarande besvär från det ställe varifrån fettet togs?

Ja Nej Vet inte

Om ja, vilka? ....
....

Har Du märkt några andra förändringar, positiva eller negativa, efter fettransplantationerna?

....
....
....
....

Hur länge behövde Du vara sjukskriven efter fettransplantationerna?
Om Du inte är yrkesverksam – hur länge behövde Du ta det extra lugnt?

....
....

Är det några andra synpunkter Du vill framföra?

....
....
....
....
....

Stort tack för Din hjälp!

Marie Wickman
Professor

Inkeri Schultz
Med dr

Anna Lindegren
Underläkare

Kliniken för Rekonstruktiv Plastikkirurgi
Karolinska Universitetssjukhuset
Stockholm

Enkät för utvärdering före och efter bröstrekonstruktion

Följande frågeformulär är framtaget för att öka kunskapen om effekterna av bröstrekonstruktion med eller utan föregående fettransplantation.

Målet är att denna kunskap i framtiden skall förbättra omhändertagandet för kvinnor som genomgår bröstrekonstruktion.

Frågeformulären hanteras konfidentiellt och är kodade så att inga personuppgifter eller namn finns kopplade till det enskilda frågeformuläret.

Tack för din medverkan!

Inkeri Schultz
Bitr överläkare, Med dr

Marie Wickman
Divisionschef, Professor

Enkät bröstrekonstruktion

Svarsdatum:..

Namn:...

Nedanstående frågor handlar om **känselförmåga** och **känselupplevelser** i brösten/bröstkorgen och besvaras genom att Du ringar in den siffra i skalan 1 - 7 som bäst stämmer överens med hur Du upplever känseln i dina bröst för närvarande.

1. Hurdan är Din förmåga att känna ***beröring*** på **böstens hud**?

Höger:	Känner normalt	1	2	3	4	5	6	7	Nedsatt känsel
Vänster:	Känner normalt	1	2	3	4	5	6	7	Nedsatt känsel

2. Hurdan är Din förmåga att känna ***sexuella känslor i brösten***?

Höger:	Känner normalt	1	2	3	4	5	6	7	Nedsatt känsel
Vänster:	Känner normalt	1	2	3	4	5	6	7	Nedsatt känsel

3. Har Du ***värk/smärta*** i brösten/bröstregionen?

Höger:	Ja, varje dag	1	2	3	4	5	6	7	Nej, aldrig
Vänster:	Ja, varje dag	1	2	3	4	5	6	7	Nej, aldrig

Följande frågor berör **sexualitet** och är känsliga och personliga, men ändå viktiga att ställa för att få kunskap om huruvida dessa områden påverkas av bröstoperationen. Ringa in det svarsalternativ som Du tycker stämmer bäst in på Dig

4. Har Dina bröst stor betydelse i din sexualitet?

Inte alls Lite Ganska mycket Mycket

5. Känner Du Dig sexuellt oattraktiv på grund av Dina bröst?

Inte alls Lite Ganska mycket Mycket

6. Har Du svårt att se Dig själv naken p g a Dina bröst?

Inte alls Lite Ganska mycket Mycket

Om Du har en partner, vänligen besvara även följande fråga:

7. Har Du svårt att visa Dig naken inför Din partner?

Inte alls Lite Ganska mycket Mycket

Följande frågor besvaras genom att Du ringar in den siffra i skalan 1 - 7 som bäst stämmer överens med vad Du nu tycker om utseendet av dina bröst.

BRÖSTENS STORLEK

8. Vad anser Du om storleken på Dina bröst?

Höger:	För litet	1	2	3	4	5	6	7	För stort
Vänster:	För litet	1	2	3	4	5	6	7	För stort

9. Är brösten lika i storlek?

Mycket olika	1	2	3	4	5	6	7	Mycket lika

BRÖSTENS FORM

10. Vad anser Du om formen på Dina bröst?

Höger:	Mycket ful	1	2	3	4	5	6	7	Mycket fin
Vänster:	Mycket ful	1	2	3	4	5	6	7	Mycket fin

11. Är brösten lika i formen?

	Mycket olika	1	2	3	4	5	6	7	Mycket lika

BRÖSTENS KONSISTENS/MJUKHET

12. Vad anser Du om mjukheten i Dina bröst?

Höger:	Mycket hårt	1	2	3	4	5	6	7	Mycket mjukt
Vänster:	Mycket hårt	1	2	3	4	5	6	7	Mycket mjukt

BRÖSTENS UTSEENDE

13. Är du nöjd med utseendet av Dina bröst?

Höger:	Inte alls nöjd	1	2	3	4	5	6	7	Mycket nöjd
Vänster:	Inte alls nöjd	1	2	3	4	5	6	7	Mycket nöjd

14. Vad anser Du om bröstens utseende när Du har kläder på dig?

Höger:	Mycket fult	1	2	3	4	5	6	7	Mycket fint
Vänster	Mycket fult	1	2	3	4	5	6	7	Mycket fint

15. Vad anser Du om bröstens utseende när Du har badkläder/BH på dig?

Höger:	Mycket fult	1	2	3	4	5	6	7	Mycket fint
Vänster	Mycket fult	1	2	3	4	5	6	7	Mycket fint

16. Vad anser Du om bröstens utseende när Du är naken?

Höger:	Mycket fult	1	2	3	4	5	6	7	Mycket fint
Vänster	Mycket fult	1	2	3	4	5	6	7	Mycket fint

17. Vad anser Du om bröstens utseende när Du har badkläder/BH på dig?

Höger:	Mycket fult	1	2	3	4	5	6	7	Mycket fint
Vänster	Mycket fult	1	2	3	4	5	6	7	Mycket fint

18. Om Du har ärr efter tidigare bröstcanceroperation - vad anser Du om ärrets/ärrens utseende?

Höger:	Mycket fult	1	2	3	4	5	6	7	Mycket fint
Vänster	Mycket fult	1	2	3	4	5	6	7	Mycket fint

BLANDADE FRÅGOR

19. Har Du svårt att hitta en BH som passar Dina bröst?

Mycket svårt	1	2	3	4	5	6	7	Inte alls svårt

20. Påverkar Dina bröst Din vilja att bada i offentliga miljöer (simhall, badstrand etc.) eller delta i gymnasik och liknade aktiviteter?

Negativt	1	2	3	4	5	6	7	Positivt

Det är viktigt att du besvarat samtliga frågor i formuläret, fråga gärna vid oklarheter.

Tack så mycket för din medverkan!